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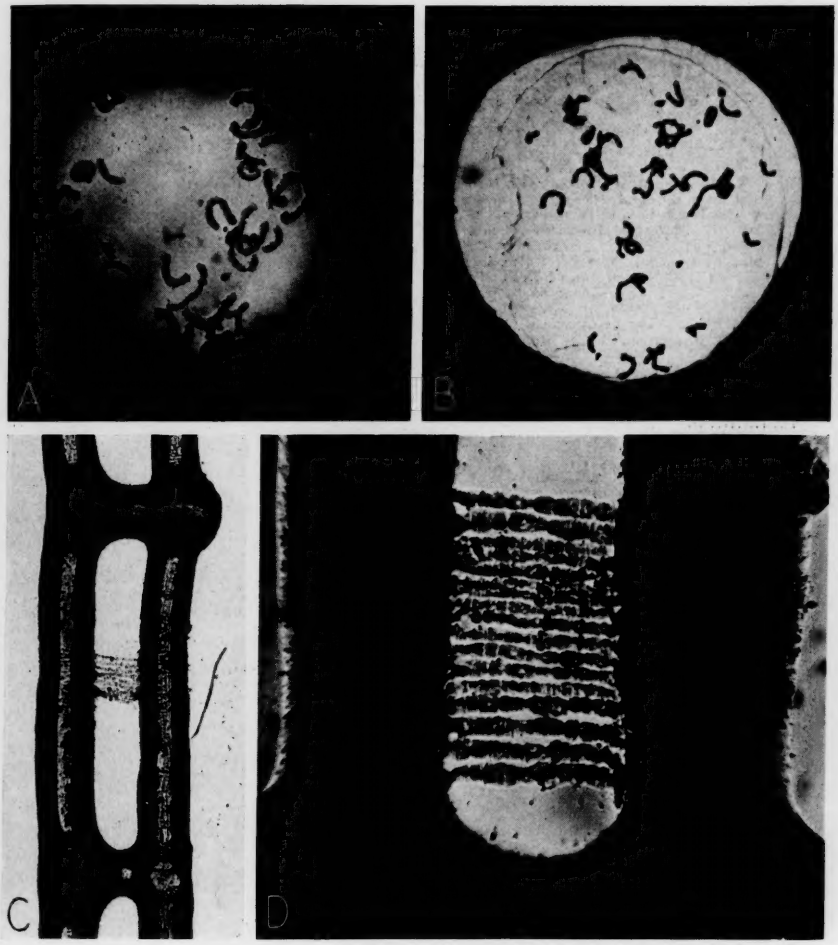

PREPARING SHEETS OF CHROMOSOMES FOR X-RAY ANALYSIS
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Colchicine-Polyploidy in Drosophila

Eye Movement in Twins

VOLUME 33 MAY, 1942 NUMBER 5

The American Genetic Association

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The JOURNAL OF HEREDITY is published monthly by the AMERICAN GENETIC ASSOCIATION at 32nd Street and Elm Avenue. Baltimore, Md., and Victor Building, Washington, D. C. Address all communications regarding editorial matters to Room 308, Victor Building, Washington, D. C. Communications regarding the business management of The JOURNAL OF HEREDITY may be addressed to the publication office, 32nd Street and Elm Avenue, Baltimore, Md., or to the Victor Building, Washington, D. C. Entered as second-class matter February 24, 1923, at the postoffice at Baltimore, Maryland. Acceptance for mailing at the special rate of postage provided for in section 1103, Act of October 3, 1917, authorized December 20, 1918.

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The

Journal of Heredity

Vol. XXXIII **MAY, 1942** **No. 5**

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A POLYPLOID AREA IN THE BRAIN OF DROSOPHILA

Figure 1

Colchicine-arrested mitoses in small ganglionic cells in a localized region of the brain (×2900). The effect of colchicine treatment on Drosophila larvae was to produce small islands of tissue having extra sets or complements of chromosomes. No large areas of polyploid cells were observed.

A CYTOLOGICAL STUDY OF THE EFFECT OF COLCHICINE

On Drosophila Melanogaster

D. C. Braungart and G. E. Ott
The Catholic University of America

THE number of cases of polyploidy recorded among animals is very limited. Moreover, there seems to be no record of polyploidy in animals experimentally induced with colchicine, although several workers have used this drug on animals and have produced mitotic abnormalities.[8, 2, 4] Among plants, however, many cases of polyploidy induced by colchicine have been reported.[1, 3, 6]

As far as is known the present study is the first experimental work dealing with the effects of colchicine on Drosophila aside from the work of Law[5] on the lethal mutation rate.

Several series of experiments were devised whereby colchicine was applied to different stages in the life cycle of *Drosophila melanogaster:*

1. Both chorionated and dechorionated eggs were subjected to concentrations of 1% and .5% colchicine. The chorionated eggs were immersed for periods of from 4 to 20 hours, while those dechorionated were treated for periods ranging from ten minutes to six hours. After removal from the colchicine the eggs were transferred to standard food and allowed to develop. A few slides of the brains and salivary glands of larvae were prepared by the aceto-carmine method. Unhatched eggs prepared for study were found to be of little value.

2. Larvae were immersed in a .5% solution of colchicine for from one to two and one-half hours. The recovery period ranged from 10 to 90 minutes, at the end of which time the glands and brains were dissected and stained.

3. In order to bring about a quick penetration of the colchicine the body walls of larvae were pierced with a sharp needle near the brain. These larvae were then subjected to a .5% and 1% solution of colchicine for from 10 to 60 minutes. The recovery period in this case was from 30 minutes to four hours.

4. In another set of experiments a modified No. 27 hypodermic needle was used to remove some of the body fluids which were then replaced by an equivalent amount of a .0013% solution of colchicine and allowed to act for from two to 18 hours. At the end of this time slides were made of the brains and salivary glands.

5. Some eggs were placed on food which was mixed with a .123% solution of colchicine. None of the newly hatched larvae lived more than 48 hours. A few slides, however, were made before all the larvae died.

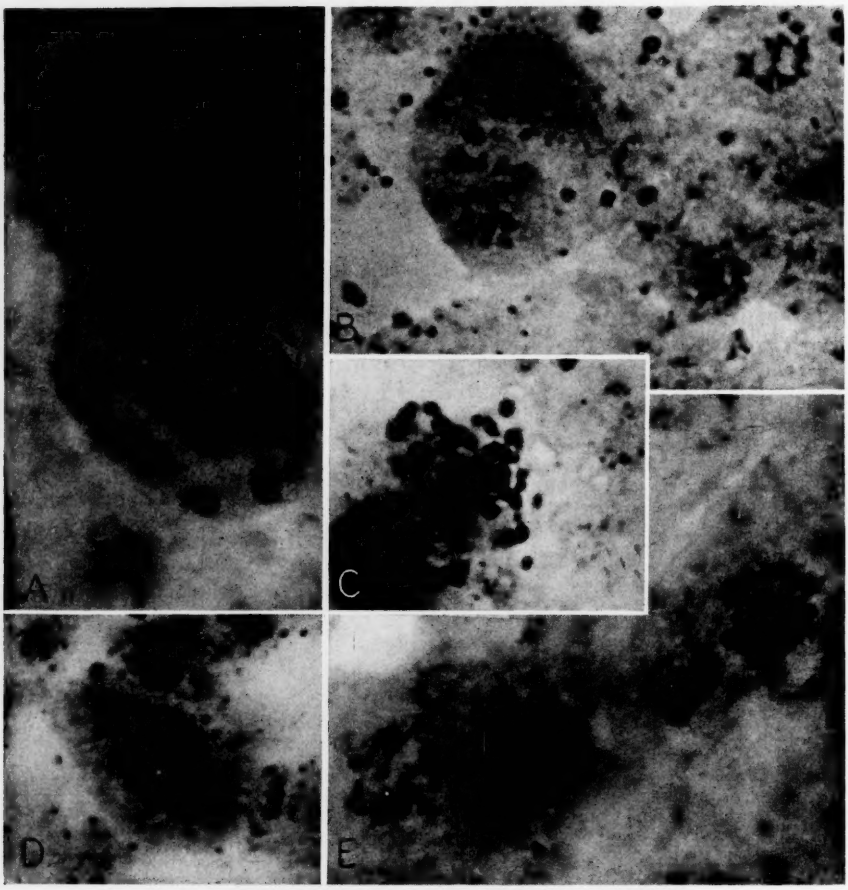
The effect of colchicine was mainly noted in a study of the dissected larval brains from experiments 2, 3, and 4 stained in aceto-carmine. The best material was obtained from the larvae injected with the colchicine. Methods 1 and 5 did not prove as satisfactory as the others.

A study of the salivary glands revealed very little change from the normal other than a slight shrinkage or contraction of the chromosomes. The cell and nuclear membranes also appeared more resistant to rupture. There was an apparent lethal effect on the chorionated and dechorionated eggs treated with colchicine, and in addition, the larval period was prolonged. This increase ranged from 6 to 11 days with an average of 7.7 days in contrast to the normal four or five day larval period.

Results

Most of the effects of the colchicine treatment were observed during mitosis. The early prophase was least affected. During mid-prophase the primary constrictions of the large autosomes were more pronounced. As can be seen in Figure 2*D*, significant configuration occurred during the despiralization process recalling the C-pairs of Levan.

In pro-metaphase there was found an abnormal shortening and thickening of the chromosomes and the absence or inactivation of the spindle. The absence



CELLS WITH INCREASED CHROMOSOME NUMBERS

Figure 2

A—Large cell showing many micronuclei (× 2900). *B*—Approximately 14 chromosomes appear in focus in the ganglionic cell (×2900). *C*—Closely grouped chromosomes visible, probably the 4*n* number (×3000). *D*—Early metaphase chromosomes showing a delayed separation of homologues and the C-pairs (× 2900). *E*—About 20 chromosomes visible in the cell at right. The two cells below it and at the left are out of focus but still show more than the diploid number (× 2400).

of the spindle may account for the chromosomes being scattered throughout the cell so that a typical equatorial plate was not formed during the metaphase. These arrested metaphases (Figure 1) showed that there was a delay in the separation of the chromatids with a consequent clumping and coalescence of chromosomes.

A true anaphase was lacking. The cells did not divide though in many cases reconstruction nuclei did appear (Figure 2*A*).

The most important single effect of the present colchicine study was the production of polyploid cells. In many of the mitoses obtained with colchicine the tetraploid number of chromosomes was undoubtedly present, though often, due to clumping and coalescence, the exact number was difficult to count.

Polyploid cells are shown in Figure 2

B, C and *E*. In all figures more than the normal diploid number may be counted.

It was hoped that all degrees of polyploidy might have been produced, with extensive areas of polyploid cells. Instead only small polyploid areas (Figure 1) were observed even after prolonged colchicine treatment. A satisfactory explanation for the fact that only small areas in the brain were affected by the colchicine treatment can not be offered at the present time.

Summary

1. The salivary gland cells were little affected by the colchicine treatment.

2. The larval period in most cases was prolonged.

3. The absence of a spindle and the lack of a typical equatorial plate was observed.

4. The chromosomes were clumped in the colchicine affected cells,

5. No true anaphase was observed.

6. Definite polyploid areas in the brain tissue showing the increased number of chromosomes were often visible.

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✦

"PRIMITIVE LITERATURE" AND EUGENICS

VAN Wyck Brooks has been writing about New England for over a quarter of a century. He has recently summed up his reactions to the American scene by authoring a biographical sketch, buttressed with copious notes, from the journal of an imaginary friend, who "died" about the time World War II began to get well under way.* Some of this "Oliver Allston's" views have a considerable eugenic interest, and an occasional insightful phrase is the more illuminating because the author is not consciously a eugenist. The book is devoted to developing Allston's views on American culture, and what makes Americans in general "tick." Allston wrote on such a variety of subjects that a detailed and orderly review of the book as a whole is hardly indicated from the eugenic point of view.

One feature of the popularization of eugenics which has long appealed to this reviewer, is that if eugenics is ever going to interest any significant number of people, it will have to be keyed into the culture pattern in which we live. There is a converse of this which is also important but not generally realized. Namely that the culture-pattern in which we live badly needs the rejuvenating action of a eugenic philosophy. Some of the sanctions which have been most effective in making the social machine tick right merrily in the past have lost much of their driving power. To give the social mechanism a renewed drive and meaning, new principles must be brought into play. And Allston shows that this reintegration has definite eugenic angles: "Primary literature somehow follows the biological grain. . . . it favors what psychologists call the 'life drive'; it is a course of regeneration that is some way conducive to race survival on what might be further called the best possible terms. I think this is the heart of Allston's notions. The great dreams, he adds, are

**The Opinions of Oliver Allston*, by Van Wyck Brooks. Pp. 309. $3.00. E. P. Dutton, New York: 1941.

those by virtue of which the race has risen, courage, justice, mercy, honor, love." If such elements are considered purely in a verbalistic context, they have little meaning. If we develop a concept of our human society as a going concern biologically, then the matter of ancestors and descendants enters in, and we see ourselves as part of the continuing pattern of life. Thus the "biological grain" of primary literature can only begin to achieve its full meaning in a eugenic context.

And Allston was not unaware that the implications of the social pattern on the survival of various human types:

> I cannot forget what Ruskin said about the business system, when it was in its heyday, at its best, when it allowed more scope for human nature than it later came to allow, with the intensification of competition. This is what Ruskin said: "In a community regulated only by laws of supply and demand, but protected from open violence, the persons who become rich are, generally speaking, industrious, resolute, proud, covetous, prompt, methodical, sensible, unimaginative, insensitive and ignorant. The persons who remain poor are the entirely foolish, the entirey wise, the idle, the reckless, the humble, the thoughtful, the dull, the imaginative, the sensitive, the well-informed, the improvident, the irregularly and impulsively wicked, the clumsy knave, the open thief, and the entirely merciful, just and godly person." Is not this a realistic statement, one that expresses in human terms the meaning of success and failure in a business world? There has been some displacement of these values: more of the "proud" and "industrious" men find themselves in the failure-class, and perhaps more of the "reckless" and "well-informed" have got into the ranks of the successful. But this general classification still seems sound, and what does it indicate in regard to the future? *That those who stand for the future,* the "sensitive," the "imaginative," the "thoughtful" along with the clumsy knaves and the open thieves, — in short, those who represent *variation,* — are those who do not thrive in a business world and therefore cannot believe in it and will not support it. It may sometimes be a personal grievance that alienates them, the fact that they, as persons, do not thrive. But neither do their virtues thrive, and can they disbelieve in these? What would become of society if they did so? That the best of the young, the makers of the future, can have no part or lot in a business world, except as the merest makeshift means of survival, seems to be a natural deduction. They feel as they do because they have to feel so (p. 123).

In another place he has this to say: "Only through health, the health of the psyche, could one find one's self by losing one's self, — only by the measure of health could one know sickness, — and one could achieve health only by the ways of health, the ways of courage, justice, love and mercy. These were the ways of the healthy psyche; and they followed the biological grain. For it was by means of these ways that the race has risen and therefore whatever follows this grain was true for human beings as whatever controverted it was in some way false. This literature favored the 'life drive,' as the psychoanalysts called it; and whatever favored the death drive was necessarily secondary."

When we consider how much of our literary tradition has developed in a biological vacuum, and when we recall the revolutionary change in man's concepts of his place in the universe in the past half century, it is not surprising, as Allston points out, that frustration and pessimism have colored a good deal of the literature of our time. While he does not use the word eugenic anywhere in the volume, this plea to consider human life and human society in terms of the biological grain is a eugenic document of real challenge and of definite importance. —R. C.

Symposia on the Relation of Hormones to Development

The Biological Laboratory, Long Island Biological Association, Cold Spring Harbor, Long Island, New York, June 10th to June 20th, 1942

As part of its policy of fostering a closer relation between biology and the basic sciences, the Laboratory invites each summer a group actively interested in a specific aspect of quantative biology, or in methods and theories applicable to it, to carry on their work and to take part in a Symposium at the Laboratory.

The Symposium this year will deal with the relation of hormones to development. As a rule the participants will be in residence at Cold Spring Harbor during all of the ten days' period. Further details, a program, and information regarding accommodations at Cold Spring Harbor may be obtained by addressing Dr. M. Demerec, the Director of the Laboratory.

TWIN SIMILARITIES IN EYE MOVEMENT PATTERNS

HAROLD E. JONES AND DAVID H. MORGAN*
Institute of Child Welfare, University of California

IN an earlier report from the Institute of Child Welfare,[4] coefficients of resemblance were compared for: (a) fraternal twins, (b) identical twins, and (c) unrelated pairs of individuals, in a series of measurements based on photographic records of eye movements.

As is well known, the act of reading is characterized by a series of ocular shifts and pauses. Photographic records taken under suitable instrumental conditions permit the measurement of the frequency and length of such pauses or fixations. Other motor aspects of reading can also be examined, including the frequency of "backward" movements of the eyes which lead to regressed fixations; this modification of the eye movement pattern commonly represents an adjustment following an inaccurate shift that has carried the eyes too far ahead on the line of print. While eye movements do not necessarily provide an index of efficient reading, the motor pattern of a good reader is frequently marked by economy, brevity, and accuracy in fixation (relatively few pauses per line, brief pauses, and few corrective regressions). Conversely, poor readers frequently show one or more of the following characteristics: division of a line into numerous fixations, accompanying a short recognition span; long pauses; and errors in placement of the eyes at the beginning or within a line, as symptomatized by regressive movements.

Table I summarizes several of the findings of the earlier study. The twins were drawn from five urban school systems in Alameda and Contra Costa Counties, California. The fraternal twins were all members of same-sex pairs; 19 of these were female, 14 male. Of the identicals, 23 pairs were female, 12 male. This is in accord with the usual finding of a somewhat larger proportion of females in twin populations.[8] The unrelated or "artificial pairs," 20 female pairs and 20 males, were obtained by matching cases on the basis of sex, school, age, mental age and reading age, from the University of California Adolescent Growth Study; the records for these latter cases were assembled by Mr. Hubert Armstrong.

As earlier studies have occasionally reported, representative samples of twins frequently are of lower average IQ than are representative samples of single-born in the same communities; in the present instance, the artificial pairs are derived from a somewhat superior selection with regard to intelligence, and they also differ from the twins in being, on the average, slightly older. It is not believed, however, that this invalidates the type of comparison that has been employed, namely, a comparison of intra-pair coefficients in variables which have only a very low relationship to either chronological or mental age, within the age range of the present groups.

For the three samples the same procedures were employed throughout. The instrument used in photographing eye movements was the opthalmograph, distributed by the American Optical Company. The reading assignments, rela-

*Acknowledgments are due to Dr. Herbert S. Conrad and Dr. Harold D. Carter for reading the manuscript, and to the staff of Work Projects Administration O. P. No. 465-03-3-61, for clerical assistance and for assistance in developing the photographic records. The collection of data was made possible through the cooperation of the school systems in Alameda, Berkeley, Hayward, Oakland and Richmond.

tively low in reading difficulty, were the standard cards (*A, B* and *C*) furnished for use with this apparatus.

With the small number of cases represented, the differences in correlation coefficients are not adequately reliable. It may be noted, however, that the correlations for fraternal twins are of the order usually found for the resemblance of fraternal twins (and of ordinary siblings) in various physical and mental traits. Can we assume that this is due to the known similarity of fraternal twins in intelligence, educational experience, and reading age? If so, we would expect that the artificial pairs would exhibit even higher coefficients, since they were chosen so as to be more similar in these respects. While the sample of artificial pairs differs in important respects from the twin sample, we may take the present evidence (from Table I) as a preliminary indication that some factor or factors other than intelligence and reading ability must be hypothesized in order to account fully for the resemblance of fraternal twins in eye movement characteristics. Eye movements in reading are not subject to ordinary observation nor can they be influenced in any direct manner by explicit training or example. It is therefore not improbable that the differences in resemblance illustrate the pervasive role of heredity in determining, through motor and central factors, the mode of expression of a learned act.

The foregoing analysis, however, would lead us to expect that in the case of identical twins intra-pair correlations for pause duration and fixation frequency would be of the order of .8 or .9, as in the case of other traits in which the resemblance of identical twins has been regarded as attributable primarily to genetic factors. While the values shown for identical twins in Table I are higher than for fraternal twins, they are lower than expected. This is not due to differences in variability; the standard deviations for fixations and pause duration are no smaller for identical than for fraternal twins. Nor is it due to unreliability of measurement, since fixations and pause duration were measured with a reliability of .95 and .94 respectively.

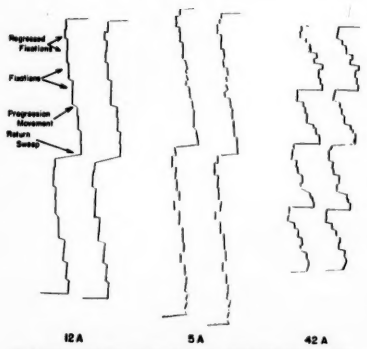
Table I is based on averages for three different sets of records. To make possible an examination of the data in greater detail, Table II, from an unpublished manuscript on file in the University of California library,[5] presents correlations for each set of eye movement records (*A, B,* and *C*) separately, and for ordinary and regressed fixations separately. All correlations were computed by the usual double entry method (rendering each correlation chart symmetrical by plotting the twin pairs twice, with reversed axes).

In Table II the median correlation for fraternal twins is .435; for identical twins .53; and for artificial pairs .105. These coefficients maintain the same relative positions when chronological age is partialled out, when corrected for attenuation, or when adjusted for differences in variability of the distributions.

Perhaps the most closely comparable study involving motor functions in twins is that by McNemar,[3] who utilized a series of tests involving manual skills. His coefficients for fraternal twins (adjusted for variability, and with age constant) ranged from .38 to .54, with a mean at .456, and are thus similar to the

TABLE I. Characteristics and Eye Movement Resemblances of Twins and "Artificial Twin" Pairs.

	No. of Pairs	Mean C.A.	Mean I.Q.	Mean intra-pair difference in IQ	*Intra-pair Correlations* Total fixations	Average pause duration
					r	*r*
Fraternal twins	33	13.01	95.0	9.8	.46	.52
Identical twins	35	12.33	96.3	5.8	.61	.73
Artificial pairs	40	13.95	113.0	3.0	.15	.35



PATTERNS OF EYE MOVEMENT

Figure 3

In reading, the eyes move in a series of shifts and pauses. These are recorded here on an opthalmograph which photographs a beam of light reflected from the cornea. Movement is from the top down, the "return sweep" representing the end of a line of type. Since all of the recordings are made at the same speed, the interval between return sweeps is a measure of the rapidity of reading. The recordings shown here are of three individuals who are members of pairs of identical twins, shown in Figure 4.

results of the present investigation. For identical twins, however, his coefficients ranged from .69 to .95, with a mean at .814.

The identical twin correlations in the present study may of course be low merely because of sampling variation. It is also possible that the basic similarity of identical twins may be registered better in some over-all characteristic (such as eye movement pattern) than in isolated variables.

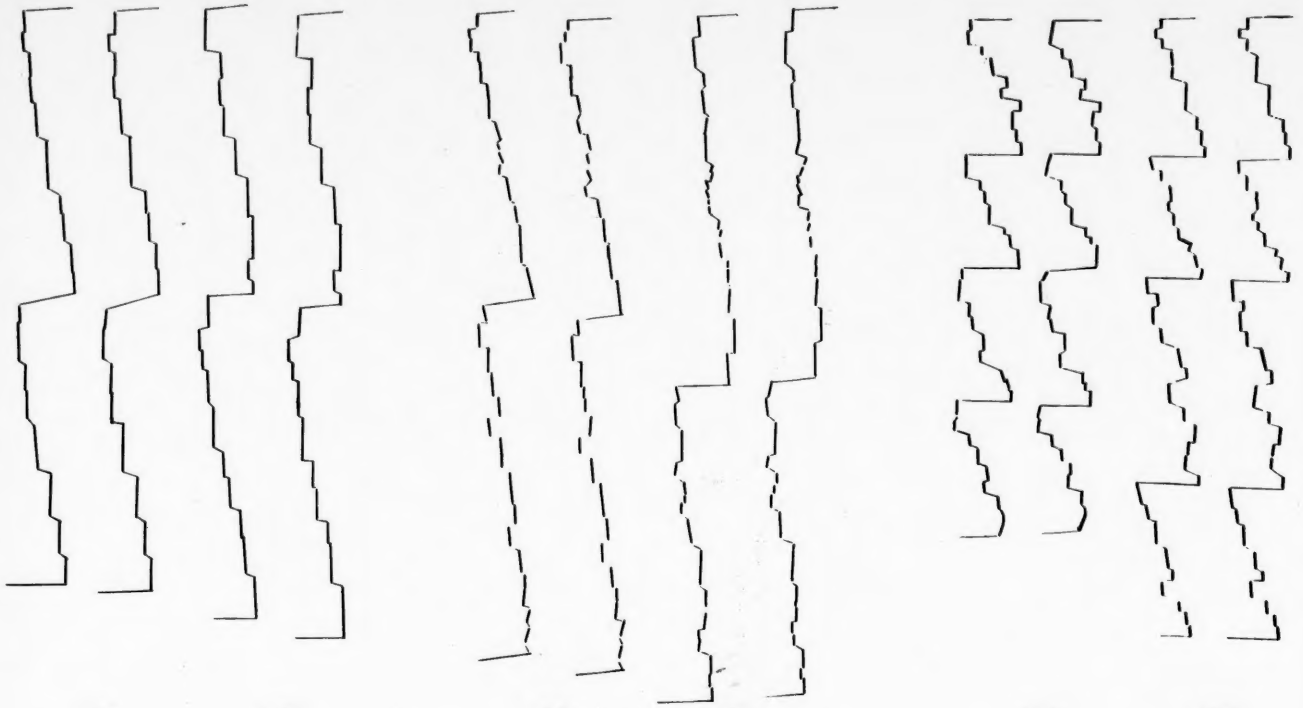
In order to test this assumption, all of the records were submitted independently to three members of the Institute of Child Welfare staff, without designation as to twin type and with no other information about the cases. They were given the instruction: "Some of these pairs of records are for fraternal, some for identical twins. On the basis of general appearance, and without regard to any specific quantitative measures, attempt to select cases which appear sufficiently similar so that on this evidence alone you would be inclined to classify them tentatively as identical twins." On this basis the first judge selected 22 pairs, approximately one-third of the total sample of twins. The remaining judges were requested to limit their selection to the same number, i. e., 22 pairs which seemed to them most similar in appearance, out of the mixed total of 68 identical and fraternal pairs. *The three judges agreed in the selection of 12 pairs of records, 11 of which were for individuals classified as identical twins in terms of all of our other criteria.**

An analysis was made of the 11 pairs of identical twins agreed upon by all judges, in order to discover factors which might possibly account for the degree of similarity noted. No such

*The diagnosis of twin type was made by Dr. Harold D. Carter[1] on the basis of techniques outlined by Siemens,[7] Dahlberg,[2] Newman[6] and others, and now quite generally accepted.

TABLE II. Correlations in Eye Movements of Twins and "Artificial Twin" Paired Controls.

		Intra-pair Correlations			
	No. of pairs	Progressions per 100 words	Regressions per 100 words	Words read per minute	Average pause duration
Fraternal twins	A 32	.52	.10	.47	.51
	B 27	.50	.19	.62	.40
	C 24	.26	.21	.53	.14
Identical twins	A 33	.41	.49	.55	.69
	B 29	.21	.54	.42	.56
	C 28	.41	.60	.57	.52
Artificial pairs	A 40	—.07	.04	.16	.14
	B 40	.07	—.20	.15	.19
	C 34	—.02	—.01	.24	.35



EYE MOVEMENT PATTERNS OF IDENTICAL TWINS

Figure 4

Here are shown the opthalmograph records of three of the pairs of identical twins recorded in this study. The differences between the pairs can be taken as an index of the variation in reading patterns which are encountered in a population. The pair of twins at the left (12*A-B*) read with longer fixations than the pair shown in the center (5*A-B*). The pair at the right show a striking similarity in type of eye movement, but one of them reads faster than the other as is shown by the difference in the length of the recording. The fastest reader (42*A*) reads about three lines while 5*B* reads one line. While a statistical analysis of the records did not show as high a correlation as might be expected, it was possible by an overall examination of the records to separate the twins into identical and fraternal with a considerable degree of accuracy.

factors were readily apparent. The pairs were spread over the entire range of the sample with regard to age, grade, and intelligence. On the other hand, the single pair of fraternal twins on which all three judges agreed was marked by a very outstanding superiority in reading efficiency; since both were at the top of the distribution in this respect, their similarity was more impressive than would have been the case in a distribution including a wider representation of superior readers.

Relaxing the standard to include cases on which at least two judges agreed, 17 pairs of records were obtained, and of these 15 were identicals. Thus we may say that the designation of identical twins from the general appearance of eye movement records was 92% "correct" in terms of the agreement of three judges, and 88% correct in terms of the agreement of two judges. For the three judges taken separately, among the 22 cases classified as most similar the percentage of identicals was 82%, 77% and 73% respectively. On the other hand, the proportion of identicals was close to 50%, or about the same as chance, in the 22 pairs of twins selected as most similar on any one quantitative measure (such as intra-pair difference in pause duration or in frequency of fixations).

Looked at from another point of view, 15 of the 35 pairs of identical twins (43%) were selected as identicals by agreement of at least two judges; only 2 of the 33 pairs of fraternals (6%) were classified by two judges as identicals, in disagreement with the other criteria. It is of course obvious that a substantial proportion of the identicals were not classified as such; 29% were left in an "undetermined" group by all three judges. A similar result, however, would be expected for nearly any single criterion of monozygosity; eye movement pattern would seem to be about as adequate an index of twin classification as any other in common use, but of course less adequate than *combinations* of criteria such as are ordinarily recommended. It should be remembered that the records employed in the present analysis are from an extremely brief sampling of reading; it may be expected that a closer agreement would be obtained by judges, and that fewer pairs would be left undetermined, if their judgments were based upon a larger series of eye movement records and upon a greater variety of reading materials.

In order to illustrate further the nature of the data used in studying eye movement patterns, Figure 3 presents patterns for three unrelated individuals. The eye movement records are produced by a beam of light reflected from the cornea; as presented here they are drawn to scale from the original photographic positives and are for the same reading selection. For each individual (cases 12*A*, 5*A*, 42*A*) records are shown for both right and left eyes. *Reading from above downward,* the vertical lines represent fixations, the small excursions to the right represent progressive eye movements (shifts from one fixation to the next) and at a few points small excursions to the left indicate regressive movements followed by a regressed fixation. At the end of each line a larger horizontal excursion occurs to the left, indicating a return sweep to the beginning of the next line. Since the film in the eye movement camera moves at a constant rate, the length of the vertical lines can be utilized to measure the pause duration; the horizontal lines vary in slope and length according to the speed and magnitude of the progression (or regression) movements. For the reading material used, the average pause duration in the twin sample was approximately one-quarter of a second, the average frequency of fixations slightly less than one per word.

Two lines of reading are shown for cases 12*A* and 5*A*, four lines for the more rapid reader 42*A*. The three individuals exhibit, characteristically, many differences in the motor aspects of reading; the result is a definite individuality in the general appearance of the eye movement patterns.

Figure 4 compares the eye movement patterns for three pairs of identical twins,

12*A* and 12*B*; 5*A* and 5*B*; and 42*A* and 42*B*. Similarities of the degree shown in these figures did not occur within the sample of fraternal twins. 12*A* and 12*B* show a fairly regular pattern with few regressions but with a relatively long pause duration. Each twin shows a regression near the beginning of each line; each twin presents several examples of extremely long fixations, and similar variations with regard to progression movements.

Twins 5*A* and 5*B* were immature readers, as evidenced by the large number of regressions and also the large number of fixations required to finish a line. Their rhythm of reading was poor, some fixations being short and others exceptionally long. Both twins show extremely long fixations at the end of the first line and short fixations at the beginning of the next. A detailed comparison reveals many points of close similarity.

42*A* and 42*B* were a pair of fairly mature readers, characterized by relatively brief fixations and occasional regressions. Both exhibit regressive fixations near the beginning of the first and third lines of the reading material. Irregularities occur for both, in pause duration and in the time of progression movements. Both exhibit an occasional lack of parallelism in the records for the two eyes. These and other minute aspects of the eye movement pattern contribute to the striking appearance of similarity. Twins 42*A* and 42*B* were able to complete four lines of reading in approximately the time required for two lines by the other two pairs.

Discussion

In terms of the so-called "habit concept," individual differences in eye movements in reading have sometimes been attributed to the direct effects of training. The results of this study support the findings of a previously cited investigation,[3] indicating that the bases for individual differences in eye movements are more complex than the habit concept implies. Intelligence and education undoubtedly receive expression in the motor as well as in other aspects of the reading process, but eye movement patterns appear also to be subtly and intimately marked by an individuality probably not wholly determined by training. We have noted that pairs of individuals in the same classrooms, with a record of attendance at the same schools, and with closely similar intelligence and reading ability, nevertheless present marked intra-pair differences in specific aspects of eye movement. Such differences are equally or even more marked with regard to general pattern. Identical twins, on the other hand, frequently exhibit a similarity in eye movement pattern nearly as great as in successive records for the same individual, and distinctly greater, on the average, than in the case of fraternal twins. This may be taken as another illustration of the influence of genetic factors, expressed not only in quantitative measures of single variables, but even more strikingly in the qualitative aspect as revealed in the total pattern of eye movement.

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METHODS FOR ISOLATING, COLLECTING AND ORIENTING SALIVARY GLAND CHROMOSOMES FOR DIFFRACTION ANALYSIS

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WITH the advance of genetics has come an increasing need for information regarding the chemical and physical properties of chromosomes. An experimental attack on this problem was hardly possible until the nature of the giant salivary gland chromosomes of Diptera was recognized (Heitz and Bauer,[1] Painter[1]). Caspersson,[1] by means of ingenious ultra-violet absorption measurements and enzymatic digestion studies, established the presence and mapped the distribution of thymonucleic acid and protein in these giant chromosomes. Mazia and Jaeger[1] and Mazia[1] confirmed and extended Caspersson's conclusion that thymonucleic acid is localized in the banded regions of the chromosomes and absent in the interband regions, and showed that the nucleic acid is attached to a continuous pepsin-resistant histone framework. The striking visible seriation of the giant chromosomes thus provides a basis not only for certain well-established genetic properties of chromosomes, such as linear arrangement of genes, longitudinal splitting and synapsis, but also for the longitudinal distribution of their main chemical constituents. This fact, together with the evidence of the fibrous nature of thymonucleic acid and histone molecules (Signer, Caspersson and Hammarsten,[45] Astbury and Bell[4]), has led to both speculative and observational attempts to deduce the molecular arrangement of nucleic acid and protein in chromosomes.

Wrinch[50] suggested that the protein is present as polypeptide chains running parallel to one another and to the longitudinal axis of the chromosome, and that the nucleic acid chains are interwoven in the protein chains at right angles to their axis. This idea was not supported by Schmidt's[43] observations on the birefringence of giant chromosomes, which indicated, like those of Frey-Wyssling[26] and Pfeiffer,[1] that both polypeptide and nucleic acid chains are parallel to the long axis of the chromosome. This conception gained support in Astbury and Bell's demonstration of the fibrous nature and parallel chain arrangement in clupein-thymonucleate, a sort of "artificial chromosome." Pfeiffer later elaborated his original conception, maintaining that his polarized light studies showed that only the protein combined with nucleic acid in the bands is in the fully-extended "β" state, while that in the interband regions is in the "α" or folded state.† The whole birefringence

*This investigation was pursued from 1937 to 1939 at the Department of Embryology, Carnegie Institution of Washington, under a grant from the Rockefeller Foundation to Professors C. W. Metz and W. T. Astbury, to whom we are much indebted. The work was continued by the junior author from 1939 to 1941 at Cambridge through the generosity of Professor James Gray. We are very grateful to Dr. Louis Maxwell, Dr. Sterling Hendricks and Mr. Vernon Mosley of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, for taking the electron photographs and for very extensive and generous assistance. We are also indebted to the Johns Hopkins University and to the Marine Biological Laboratory (Woods Hole) for facilities, and to Dr. A. Mirsky and Dr. M. Huggins and colleagues of the senior author for aid with the manuscript.

†A similar structure has been inferred from chemical evidence by Calvin and Kodani (*Proc. Nat. Acad. Sci.* 27:291-300. 1941).

work, however, has been weakened considerably by Caspersson's[14] demonstration of certain limitations of the technique and of the great danger of artifact due to the tendency of the fibrous molecules of nucleic acid to become aligned artificially. Caspersson was unable to obtain any evidence for fibrous structure in giant chromosomes, using a very sensitive method involving ultraviolet dichroism. As a matter of fact, in much of the birefringence work there has been a tendency to draw conclusions about molecular orientations which are unjustified except with pure substances. In the usual heterogeneous biological material, birefringence is likely to be due simply to orientation of miscelles ("form" birefringence; cf. Schmitt[44]) rather than true "crystalline" anisotrophy. The latter is more reliably detected by diffraction methods.

In view of the confused state of knowledge concerning the chemistry of chromosomes, further information on their molecular constituents and intermolecular linkages would be of great value. Astbury[1] and others have had conspicuous success in analysing the molecular structure of fibrous proteins by means of X-ray diffraction. The present paper describes several attempts to apply this method, and the analogous technique of electron diffraction,* to the study of giant chromosomes. It should be stated at the outset that this investigation, like the similar study of Harrison, Astbury and Rudall[28] on embryonic tissue, has thus far yielded only inconclusive results. These are presented at this time because the war has interrupted the work, and a progress report may be of help to other investigators. In addition, the methods developed in this study for isolating nuclei and chromosomes are of potential use in other types of chromosome work.

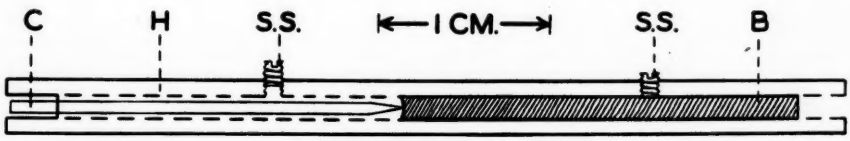
Biological fibers ordinarily give diffraction pictures varying from spots symmetrically arranged with respect to two axes ("fiber" photograph), to somewhat less informative patterns of concentric rings ("powder" photograph). The former occurs when the unit crystallites of the fiber are oriented with respect to the fiber axis; the latter when the crystallites are more or less completely disoriented. From the spacings of the spots or rings in the pattern, some information on the dimensions and linkages of the molecules composing the fibers can often be deduced. If chromosomes have a fibrous structure, similar, for example, to that formed by the polypeptide chains in silk fibroin, an X-ray photograph of a bundle of straight parallel isolated chromosomes might be expected to give a fiber pattern. A photograph of a mass of whole nuclei, on the other hand, should give a powder pattern of concentric rings, because the chromosomes in each nucleus are of course present in all possible orientations. This leaves out of account non-chromosomal material, such as sap, membrane and nucleoli. However, the work of Doyle and Metz[18] and of Buck[10] indicates that these occupy only a small proportion of the total volume of most salivary gland nuclei.

Material

Professor Astbury had estimated that the minimum necessary amount of chromosomal material for X-ray diffraction study would be a cube, 100μ on an edge. Since this is a large mass, in cytological terms, it seemed mandatory to use the largest chromosomes available. Moreover, the technical difficulties involved in orienting individual chromosomes seemed so great that the problem of preparing masses of whole nuclei was attacked first.

Several methods for the isolation of nuclei from cells and tissues have been described previously. Marshak[34] reports that Smith separated skin and tumor nuclei by acetic acid treatment in 1856, and that Ploz and Ackerman obtained nuclei from avian erythrocytes by hemo-

*Electron diffraction is not to be confused with electron microscopy. In the former, a narrow beam of electrons passes through the object and breaks up into various diffracted beams; in the latter, beams of electrons are bent by "magnetic lenses" and form an image of the object comparable to that formed by visible light in ordinary photomicrography.



APPARATUS FOR CENTRIFUGING NUCLEI IN FINE CAPILLARY

Figure 5

H—Tubular brass holder; *C*—thin-walled capillary in which nuclei are placed; *B*—block for adjusting position of capillary; *S.S.*—set screw.

lysis. Crossman[17] used five per cent citric acid to isolate nuclei from muscle, and Marshak used it on liver and tumor, showing, incidentally, that the effect was due to the hydrogen, rather than to the citrate, ion. Stoneburg[46] combined Crossman's method with Miescher's[38] peptic digestion method, but his claim that the "nuclei are left histologically undamaged" appears questionable in view of Mazia's work on pepsin digestion of salivary gland chromosomes. Behrens[6] devised a method, used also by Gulick,[27] in which the nuclear material is centrifuged out of finely powdered tissue suspended in organic fluids, but the manifest difficulty in being sure that no extranuclear contamination is present, plus the loss of structural integrity of the chromosomes, made this method of doubtful value for use in the present investigation. Duryee[20] describes methods applicable to the isolation of the very large germinal vesicles of amphibian eggs.

In this investigation, salivary glands from mature larvae of *Chironomus tentans* and *C. plumosus* were used. The nuclei in these glands averaged 75 to 100 μ in diameter, and the chromosomes $20 \times 20 \times 150$ μ. The glands were dissected out into larval blood or Ringer* and subjected to one of the treatments described below.

Isolation of nuclei from unfixed glands

Theoretically, of course, it would be desirable to keep the nuclei and chromosomes in the "normal living" state, but for X-ray analysis this possibility must be discarded at the outset, since X-rays themselves cause extensive changes in living cells, and because the technique requires material containing a minimum of water. Moreover, in the present project some preservative was necessary to guard the material from decay during the journey to England and the prolonged exposures in the X-ray camera. Finally, it was found in actual practice that dissection of cells in "living" glands (i.e., untreated save for dissection into blood or Ringer) caused an immediate, marked and irreversible shrinkage of the chromosomes and that dissection of these shrunken chromosomes was infeasible because of the extreme stickiness and plasticity of the protoplasm (Vonwiller and Audova,[1] Stefanelli,[1] Glancy,[1] Buck[12]).

Two methods, however, were discovered for isolating nuclei from unfixed, though not necessarily "living," glands. In the first, the glands were soaked for an hour or two at room temperature in distilled water. The nuclei swelled so much that they could be shaken loose from their cytoplasmic sockets with fine glass needles, and they were so highly hydrated that they appeared empty. It was discovered that if a gland which had ceased swelling in a drop of distilled water was transferred to a fresh drop, it swelled further; and conversely, that if a freshly dissected gland was placed in a drop in which another gland had soaked, the new gland failed to swell. Its cytoplasm, however, altered sufficiently to permit the isolation of nuclei. These facts suggested that something

*Composition: 5.55 g. NaCl, 0.22 g. KCl, 0.44 g. $CaCl_2$ per liter.

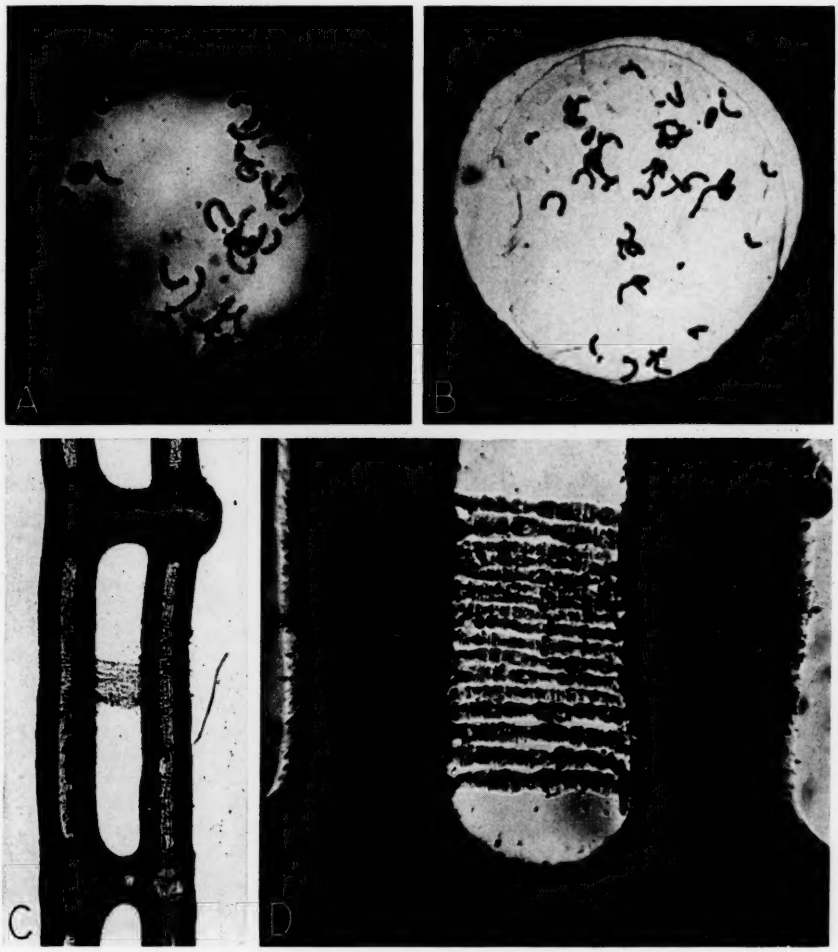
had dissolved out of the gland; the further fact that heating such a drop produced a flocculent precipitate, suggested that the dissolved material contained a protein. This surmise led to the second method (Melland[35]): The glands were soaked for two or three hours in a 0.25 per cent solution of dried egg albumin (Merck) at 5° C. after which the nuclei were dissected out by hand on an ice-cooled stage. The chromosomes were not visibly distinguishable from those in the living animal (Buck[10]). Moreover, they showed the same plasticity and stickiness which is characteristic of the chromosomes in freshly dissected glands in isotonic Ringer (Buck[12]), and they even retained to some extent the ability to swell and shrink reversibly in response to osmotic changes, which has been shown by Doyle and Metz *in vitro* and by Buck and Boche[13] *in vivo* to be a prime characteristic of living chromosomes. The mechanism of the action of albumin is very obscure. Perhaps the chromosomes are partly "stabilized" by the formation of an ovalbumin-nucleic acid complex similar to that described by MacInnes and Longsworth,[32] whereas the cytoplasm is swollen and detached from the nuclei in a manner analogous to the effect of distilled water. This implies, however, a penetration of cell and nuclear membranes by the albumin.

Like the nuclei in "living" glands, neither the nuclei isolated after distilled water treatment, nor those from albumin were satisfactory for "powder" photographs without further treatment, because with time, their chromosomes underwent progressive changes, possibly indicating denaturation of their proteins, and their stickiness made them difficult to collect in large numbers. The techniques are mentioned, however, because they should be eminently suitable for physiological investigations, and for chemical analyses in which nuclei are desired which presumably have undergone a minimum of chemical change.

Isolation of nuclei and chromosomes from fixed material

The intractability of nuclei isolated from fresh glands made it necessary to find methods of fixation which would permit isolation of nuclei and single chromosomes and yet not cause too drastic chemical changes.* Crossman's citric acid technique was tried on *Sciara* glands, where nuclear changes can be measured accurately.[13] It not only caused over 40 per cent shrinkage of the chromosomes, but made the cells rubbery and the nuclei less dissectable than before. Three reagents were found to be satisfactory: (1) acetic acid; (2) formalin; (3) osmium tetroxide vapor. All three reagents produce a sequence of changes, in which first the cytoplasm becomes brittle, permitting the isolation of nuclei which are intact but in which chromosomes are too sticky to remove whole; then, with longer treatment of the gland, the nuclear membranes also become brittle and can be cracked open and single chromosomes extracted. Later still, the chromosomes themselves become brittle. The proper duration for a given treatment varies considerably with material, concentration of reagent and temperature. With 45 per cent acetic acid, immersion of the gland for an hour or so at room temperature was ordinarily sufficient to make the isolation of chromosomes possible. With formalin, an immersion of thirty to sixty hours at 14° in a 10 per cent solution was the most satisfactory treatment in terms of preservation of chromosome structure and of ease and speed of dissection of intact nuclei (fifty to eighty per hour being possible). With osmium tetroxide, the glands were put into a hanging drop of isotonic Ringer on a coverglass which was then sealed with Mayer's albumin over a drop of two per cent solution in a deep-welled slide. Exposure for ten to fourteen hours at 6° C. ordinarily gave easily dissectable nuclei and chro-

*So little is known of the chemical structure of chromosomes that judgment of what constitutes "injurious" treatment must be based on analogies, particularly with protein denaturation. Even there, much confusion exists as to the types of denaturation, reversibility, etc.[48]



METHOD OF PREPARING AND ARRANGING CHROMOSOMES

Figure 6

A—Chromosomes in water after dissection from glands treated with osmic acid vapor (× 50). *B*—Chromosomes similar to those in *A*, dried on a thin collodion membrane stretched across a hole in a thin brass sheet (× 50). *C*—Framework of DeKhotinsky cement for holding oriented chromosomes (× 30). Note the chromosomes stretched across the center of the frame. *D*—Seventeen *C. plumosus* osmium-treated chromosomes in water, stretched between cement supports (× 130). Such sheets of chromosomes were piled on top of each other to produce a solid block of chromosomes for x-ray diffraction analysis.

mosomes. With each reagent, after optimum treatment, the gland was transferred to distilled water and dissected by hand under 25 to 50 diameters magnification of a dissecting binocular. The nuclei were isolated with fine steel needles, the chromosomes with fine glass needles.

Acetic acid, although widely used in cytological fixation, causes drastic shrinkage of chromosomes (Doyle and Metz) and is also known to denature

some proteins. The acetic acid technique was therefore judged the least suitable of the three for X-ray analysis and was little used. It should, however, be satisfactory for collecting nuclear or chromosomal material for many types of chemical analysis.

The chemistry of the formalin protein reaction has been fairly well worked out* and indicates that formalin should be fitted for X-ray and many chemical analyses. In addition, Doyle and Metz, Ries,[41] Zeiger,[51] and others report that it causes hardly any visible changes in nuclei.

The chemical effect of osmium tetroxide,† though less well understood, appears to be the mildest and least disruptive of any known fixative, and should be favorable for diffraction analysis. It has the additional possible advantage that it contains very heavy atoms, which of course have a high absorption for X-rays. They might thus enhance the diffraction pattern of the (hypothetical) protein framework of the chromosome, if they attached at regular intervals. Finally, it has long been regarded as a superb cytological fixative (Flemming,[25] Meyer,[37] Baker,[5] Doyle and Metz, etc.), which fact Zeiger ascribes to lipoid-stabilizing properties. At any rate, it leaves salivary gland chromosomes scarcely distinguishable from those in living nuclei. The preservation is so faithful, indeed, that individual chromosomes and even individual bands are readily distinguishable after dissection, and it is thus feasible to make collections consisting only of one particular chromosome from each nucleus (see Figure *7B*) or even of a part of a chromosome. In addition, the chromosomes retain a high degree of elasticity (Buck[12]).

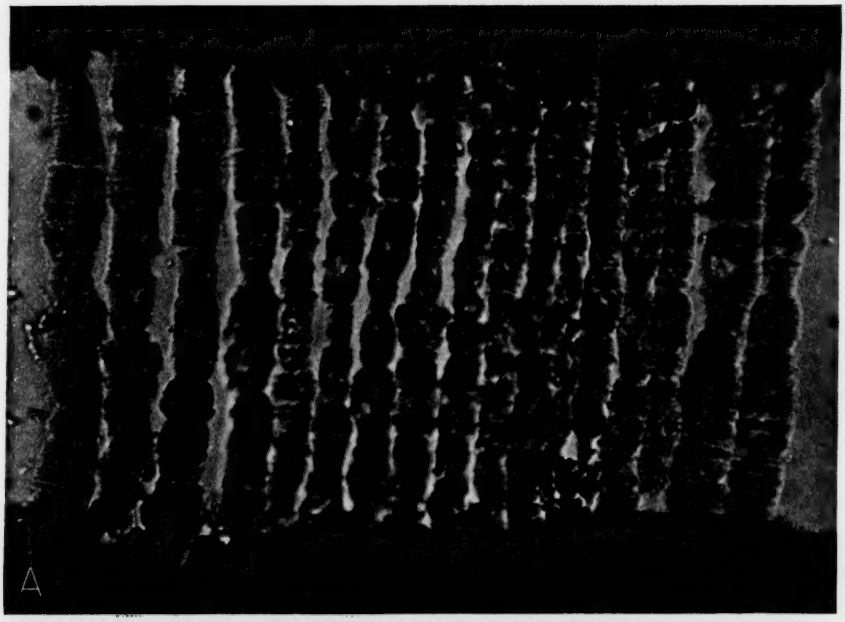
Methods for collecting and mounting nuclei

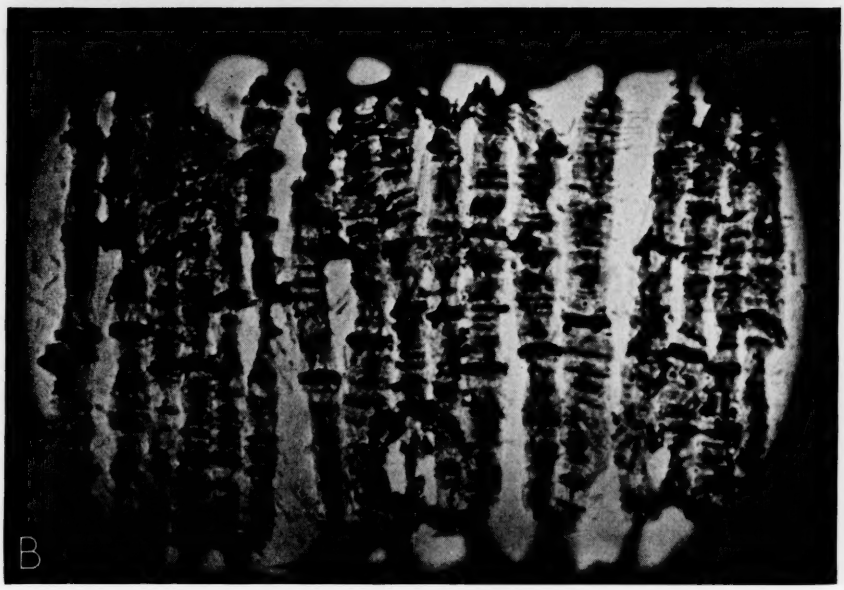
Nuclei were collected and transferred by means of micro-pipettes of 0.1 to 0.3 mm. bore. In preparations in which the nuclei were not sticky, transfer was much facilitated by the use of constriction micro-pipettes (such as are used in transplantation work in *Drosophila*) which act as filters permitting the transfer of large numbers of nuclei in a minimum of fluid.

For X-ray photography, the masses of isolated nuclei were either transferred to a thin collodion membrane and left to dry, or centrifuged into the tips of extremely thin-walled borosilicate or Pyrex glass capillaries, 0.3 to 0.5 mm. in diameter, furnished by Professor Astbury. (Lithium glass, the most permeable to X-rays, proved too soluble in

*It is well known that formalin stabilizes polypeptide chains by combining with free amino groups, but it is uncertain whether other more extensive or disruptive alterations also occur. To cite only a few papers from an extensive literature, Fischer[24] and Blum[9] found that formalin hinders the denaturation of proteins by heat. Reiner and Marton[40] reported that formalin has seemingly contradictory stabilizing and coagulating effects on albumin, from which they postulated a dissolution of the peptide bond. Ries mentioned similar effects of formalin on albumin and gelatin. On the other hand, Chow and Goebel,[15] Dubos,[19] Eagle[21] and Ross and Stanley[42] found that formalin inactivates pneumococcus antibody, diphtheria antitoxin and tobacco mosaic virus, but that the inactivations are readily reversible. A partial reversibility was found also by Holden and Freeman[30] and Wadsworth and Pangborn[49] in the reactions of amino acids with formalin. The latter authors postulate a three-step reaction. Anson[3] reported that carboxypeptidase acts even in the presence of formalin. Lauffer[31] observed unchanged stream birefringence in virus after formalization. In sum, these results point to a loose and non-disruptive combination of formalin and protein and indicate that formalin should be a favorable reagent for preserving protein structure for X-ray analysis. However, the well-known propensity of formalin for polymerization points to the need for caution, since Clark and Rowan[16] have shown that in X-ray photographs of formalized protein, spurious rings arise from the polymer.

†The effects of osmium tetroxide solution and vapor on proteins have been investigated extensively (Fischer,[24] Monckeberg and Bethe,[39] Mann,[33] Baker,[5] etc.). In general its effect is to convert protein solutions into a stable, insoluble, homogeneous gel, accompanied by practically no precipitate and by increased resistance to precipitation by alcohol and heat (Berg[8]). Later there may be a secondary effect on fatty substances, involving a partial reduction of the tetroxide (Hoerr[29]). In salivary gland chromosomes the only perceptible effect is a reduction in surface stickiness, which has led to the suggestion that surface denaturation occurs (Buck[11]).





ORIENTED CHROMOSOMES

Figure 7

A—Same as Figure 6*D* (× 335). Note delicate internal structure of those chromosomes which are in focus. *B*—Plate of *C. plumosus* chromosomes similar to that in *A*, after drying on glass. Note preservation of internal structure. This plate was made from seventeen identical chromosomes (identified by the dark "nucleolar" band), one from each of seventeen nuclei (× 280).

water.) In centrifuging, the capillary was held in a brass holder (Figure 5*H*) constructed to fit the hematocrit head of the high speed Bausch and Lomb centrifuge (9,000 r.p.m., 7 cm. radius). The holder had a notch in the centripetal end so that the transfer of nuclei and removal of excess fluid could be observed without moving the capillary. Adjustment for varying lengths of capillary and accidental chipping of the very fragile lip was made by shifting the cylindrical block (*B*) in the holder after which it was locked in place with one of the set-screws (*S.S.*).

X-ray diffraction photography of whole nuclei

Four preparations of intact nuclei were photographed by Professor Astbury: the first consisted of 800 nuclei isolated from distilled water, treated with 20 per cent acetic acid to reduce stickiness, and dried on a very thin collodion membrane stretched across a glass loop; the second included 750 nuclei, isolated by the osmium tetroxide technique, centrifuged into a borosilicate capillary and allowed to dry; the third and fourth were of 1,000 and 6,500 nuclei, respectively, isolated by the formalin technique, shrunk somewhat in 25 per cent formalin to reduce water content, centrifuged into the tips of capillaries and sealed off in a minimum of fluid. Preparation number four was photographed in both a semi-dry and wet state.

The results of the photographs were that the first preparation gave no diffraction pattern at all, and that the other three gave only diffuse patterns due to the glass. It was not possible to tell from the pictures whether the failures were due to too drastic fixation, to interference from the mounting media, to insufficient material, to an insufficient proportion or lack of crystallites in the material, or to possible disturbing effects of the non-chromosomal material (nucleolus, membrane, etc.) in the nuclei. In order to eliminate the last possibility, only isolated chromosomes were used in succeeding work. In order to insure that sufficient material was present, the method of electron diffraction was next employed, because it has the advantage over X-ray diffraction that it requires only extremely small amounts of material.

Electron diffraction photography of chromosomes

To offset the above-mentioned advantage, the electron diffraction method has the serious disadvantages that the material must be photographed *in vacuo* (i.e., bone dry) and that spacings greater than a few Ångstroms cannot be detected without special methods. In addition, both diffraction methods have the disadvantage that there is no simple way of "scanning" the field and directing the narrow beam through a selected region. This difficulty was of no account with X-ray diffraction, where the problem was to get a large and thick enough pile of chromosomes, but with electron diffraction the material must be less than one micron in thickness, which means that the chromosomes had to be distributed in a single layer, with the consequent large risk that the beam would miss them altogether.

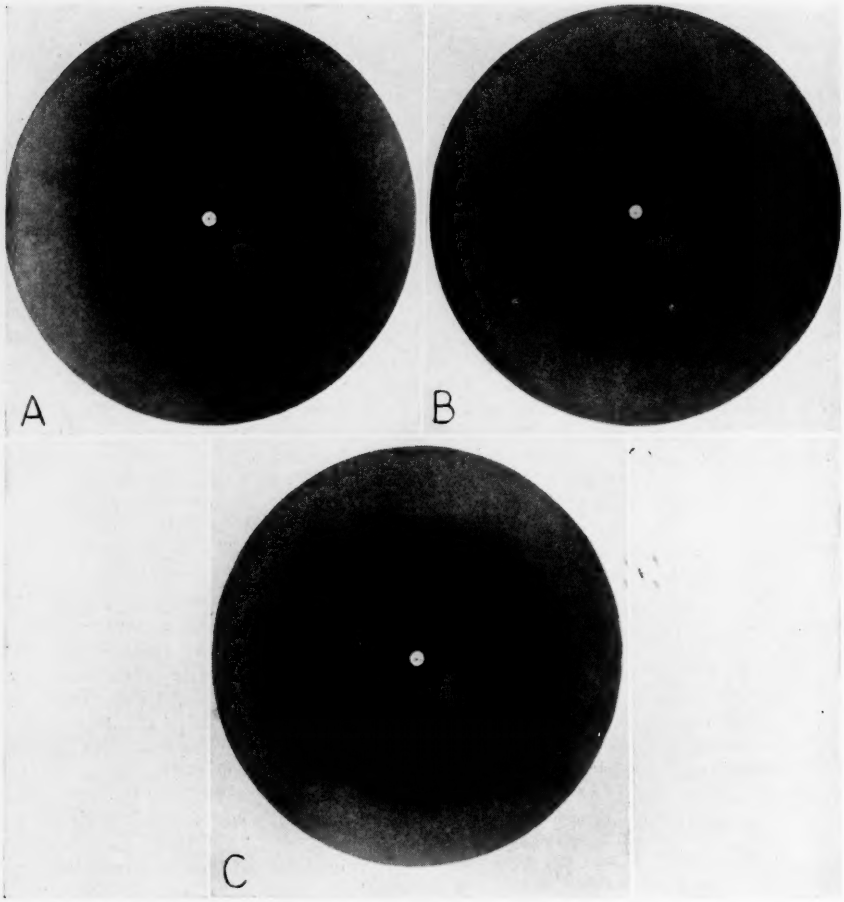
Chromosomes isolated by the osmium tetroxide technique and thoroughly washed in distilled water were used, and four types of preparations were investigated. In the first (Figure 6 *A* and *B*), chromosomes were dried on very thin (10^{-5} cm.) collodion membranes and photographed with a transmitted beam of electrons (40 kv.). None of these photographs showed any spacings which were not also on the control negatives. This was due perhaps to difficulty in scanning, but a larger number of chromosomes could not be used because the membrane usually ruptured, due to strains set up by the chromosomes in drying. In the second method, larger areas of the membranes were covered by sprinkling them with a coarse powder obtained by grinding hundreds of dried chromosomes between two pieces of optically flat plate glass. Here again the photographs were negative. In the third method, chromosomes were dried on sheets of polished chromium-plated brass

(to which they do not stick when dry, as they do to glass) and then pried up and cemented across minute holes in a sheet of brass. Although these flat dried chromosomes were only about 1 μ thick, this was too thick to transmit the electron beam and the chromosomes were vaporized by the absorbed energy. In the fourth method, the chromosomes were dried on the highly polished hemispherical surfaces of minute copper holders, similar in shape to collar buttons. A beam of electrons was directed tangentially to the surface with the hope that either transmitted or partially reflected electrons might pass through some protruding edge or end of a chromosome. Several exposures on three preparations were made, none of which differed from the controls except one, which had an extra ring at about 3.4Å. This is an interesting result because of its possible relation to both the polypeptide residue spacing and that of nucleotides (ca. 3.4 Å).[4] However, microdissection experiments (Pfeiffer,[1] Buck[12]) indicate that relaxed chromosomes, such as were used here, are probably not in the "fully extended" (β) state corresponding to the extended peptide spacing of 3.4 Å. Moreover, the result could not be repeated, due perhaps to the scanning difficulty already mentioned, and thus must remain merely suggestive.

X-ray diffraction study of oriented chromosomes

The results of the foregoing work seemed to leave only the alternative of preparing a "block" of parallel chromosomes, which, as previously explained, might be expected to give a "fiber" pattern. The method devised was as follows: two threads of DeKhotinsky cement (Cenco "Sealstix") 0.1 mm. in diameter were laid parallel about 0.3 mm. apart on a glass slide and short strands ("rungs") of similar thread were laid across the two fibers to form a sort of ladder. The slide was then heated so that the threads flattened and ran together to form a continuous framework (Figure 6*C*). A drop of distilled water containing chromosomes isolated by the osmium tetroxide vapor technique (the formalin technique could have been used instead) was placed between the threads and a single chromosome was manipulated by hand with fine glass needles until one end could be pressed into the cement on one side. The other end was then captured between the two needles and stuck into the cement directly opposite the first end, stretching the chromosome from 30 to 50 per cent. (It would have been desirable to stretch the chromosomes about 100 per cent, on the assumption that polypeptide chains would then be extended, but this was not possible because they usually tore loose from the cement). Other chromosomes were oriented in the same way, parallel to and touching one another until a "plate" of 10 to 15 was completed (ca. one to three hours; Figures 6*D* and 7*A*). The cement was then gently pried loose from the slide, and the plate transferred in plenty of distilled water to another slide and left to dry. Figure 7*B* shows that the chromosomes retain a surprising amount of visible structure after drying. After the plate was dry the ends of the chromosomes were cut loose from the cement, which was removed, leaving the chromosomes lightly adhering as a unit to the glass. Additional plates were made, transferred and dried on top of and parallel to the original so that a solid "block" was built up. The blocks were built up on glass instead of on chromium because the advantage of being able to see and orient the chromosomes by transmitted light outweighed the loss of a few chromosomes from the bottom plate when the block was mounted for photography. On an average day, about three plates (40 chromosomes) were mounted.

The largest block of parallel chromosomes prepared contained 87 plates (1,253 chromosomes) and was approximately 0.25 mm. square and 0.05 mm. thick. This was completed early in 1941, by the junior author, mounted on a very thin borosilicate glass membrane, and photographed (during an air-raid) by Professor Astbury. The result is presented in Figure 8*A*. As compared with



DIFFRACTION PHOTOGRAPHS OF CHROMOSOMES, GLASS, AND CEMENT

Figure 8

A—X-ray diffraction photograph of block of 1535 parallel chromosomes mounted on glass film. The inked-in arcs indicate the positions of two faint rings, parts of which are visible on the original picture in mirror-image position to the inked-in region. In all X-ray pictures, the technical conditions during photography were about as follows (further details in Melland[36]): Cu K_α radiation ($\lambda = 1.54$ Å); slit varying from 1/10 mm. to 1/2 mm.; plate distance 4 to 7 cm.; exposure, averaging 60 hours. *B*—X-ray diffraction photograph of glass membrane upon which chromosomes were mounted, showing no rings. *C*—X-ray diffraction photograph of drawn-out DeKhotinsky cement. (Four hour exposure.)

the glass control (Figure 8*B*) the chromosome preparation shows the presence of some crystalline material. In the original picture, parts of two faint rings of about equal density can be seen. Concerning these rings Professor Astbury writes: "Florence [Dr. Bell] has taken several more photos of the chromosome preparation and DeKhotinsky. The photo of a part of the glass film [Figure 8*B*] near to the chromosomes (same exposure and everything) showed only the darkening near the center, without the two vague rings. DeKhotinsky [Figure 8*C*] gives at least two rings, one of which seems to coincide with one of the chro-

mosome rings, but there is not sufficient agreement to conclude that the latter are from the cement alone . . . so they must arise either from some component of the chromosomes themselves, or from a decomposition product, or from the fixing agent [unlikely, since no rings showed in the photo of osmium-treated whole nuclei]. It's impossible to say which, of course, and as they are not oriented, and are not protein or a nucleic acid we know, nothing further can be said." It is by no means clear, however, that the technique has been explored adequately. More sensitive methods (Fankuchen[22]), different wavelengths, or different methods of preparation may permit a more definite conclusion.

Summary

Two methods for isolating salivary gland nuclei from unfixed glands and three methods for isolating nuclei and single chromosomes from fixed glands are described. Techniques for collecting these nuclei and chromosomes and mounting them in various ways for X-ray and electron diffraction photography are described. The photographs taken to date have not justified any definite conclusions regarding the structure of salivary gland chromosomes, but the technique has not been adequately tested. It is pointed out that the methods may be of value in physiological and biochemical investigations, and in electron microscopy.

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THE STATUS OF HEREDITY-ENVIRONMENT STUDIES ON THE DEVELOPMENT OF HUMAN INTELLIGENCE

STUDIES of twins and foster children are really the only procedures that have contributed anything crucial to the problem of heredity and environment as they influence the development of individual differences in human intelligence. It is not that single children or children growing up in their own homes *could* not contribute information of considerable value if we were able to design experiments from which we could estimate the potency of environment to make or mar. Except in a few situations where environmental deprivation has been almost pathological—such as children growing up in the meagre and restricted environments of canal boats or in hidden mountain villages in the Appalachians — there is practically nothing in the experimental literature that will hold water except the studies of foster children and of twins, particularly the studies of twins reared apart. Hence it is important from time to time to take stock of how research in this field has been progressing.

There has been too much loose thinking regarding even the twin and foster child material, and the idea has got abroad that some of the studies of twins and foster children are actually conflicting in their implications. To get "the record" straight, the Social Science Research Council recently requested R. S. Woodworth—widely recognized for his integrity and for his resistence to "fractious factions"—to go over existing studies with a fine-tooth comb, and to pull out what there was in the way of tested, sifted, facts and legitimate inferences, so that these could be viewed in the cold light of reason by all who can and do appreciate the truth.

Some of Woodworth's conclusions are rather surprising—yet the facts which he collates and analyses seem to make them quite irresistible. We have become so accustomed to seeing data on identical and fraternal twins presented in the form of correlations or "average differences between pairs," that we are startled by Woodworth's flat-footed statement that the usual identical-fraternal comparisons based upon traits which develop in a culture and are somewhat influenced by social environment, are mostly worthless for evaluating the relative contributions of nature and nurture. Comparisons between pairs of identical twins do show, to be sure, the extent of the effect of environment when the individuals of a pair are reared together, since obviously the failure of two individuals who are genetically identical to develop a particular trait exactly in parallel must be attributed to the kind of "random" deviation in environment which is always superimposed even when life-patterns are very similar. The fact that fraternal twins show larger differ-

*WOODWORTH, ROBERT S. Heredity and Environment. A critical survey of recently published material on twins and foster children. Pp. $X + 91$. New York: Social Science Research Council, 1941.

ences than identical twins is not, however, evidence for much of anything, since it can easily be demonstrated that the environments of fraternal twins differ in many respects more than do the environments of identical twins. The fact that the environments of the fraternals *are* more different can quite plausibly be attributed to their different genes. This hardly throws much illumination on the problem, since it is necessary to have similar environments in all essential respects before we can conclude that developmental differences are first of all due to gene potentialities in the organism. Woodworth comments that a comparison of fraternal twins with ordinary siblings might really be of more value than a comparison of identical twins and fraternal twins, since fraternal twins are averagely no more alike in their genes than are siblings. Any greater developmental similarities in fraternal twins as compared with siblings could therefore be attributed to a somewhat greater similarity in effective environment by virtue of the fact of being twins.

Twins Reared Apart

Woodworth looks for the most crucial results in the material based upon identical twins reared apart from infancy. Although the number of cases so far on record is not large, the results are clear-cut as far as they go. When environmental differences are large this is reflected in distinct differences in intelligence as measured in a variety of ways. Where one member of an identical pair has only two or three years of elementary school and his twin attends college and even graduates, the difference in I.Q. may be expected to amount to 20 points or more. That the difference represents more than a mere technical ability to make scores on an intelligence test is strongly suggested by the other facts in the developmental histories of the twins. Not only in level of vocational adjustments, but also in many other ways of meeting the exigencies of life, the twins with more education seem to have an advantage over their co-twins with meagre education.

Of course one may say this is only what might be inferred by common sense, but there have been in the past some rather radical statements by the "nativists" concerning the inviolability of the I.Q. against almost any sort of assault short of a lesion of the central nervous system. It has likewise been claimed that the hierarchy of intelligence as found in adults who have attended college, high school, or only elementary school, means merely that pupils of higher I.Q. reach a higher rung in the school ladder. To finish high school or college, the proponents of this view claim, is a certification of high intelligence, but may not affect the I.Q. itself. No doubt this is partly true, but the data on twins reared apart show unequivocally that it is only partly true. Education or the lack of it can affect the I.Q. by appreciable amounts for better or for worse. There are other less intellectual traits of temperament and social attitudes which environment also influences in a demonstrable way, but Woodworth's treatment of the data is largely confined to influences upon the I.Q. — genetic and environmental. He — and the reviewer — therefore only hint at the fascinating aspects of the twin case histories which bear upon the complete individual as he functions in his social group, in his family, in his vocational niche, etc.

Foster Child Studies

Moving over to the problem of foster children, Woodworth concedes that some extremely interesting and suggestive material has been obtained through the study of children separated from their own parents in early life. He concludes, however, that nothing very precise can be obtained in this way, at least until a much more rigorous experimental design has been achieved than has been possible in the past. We do not have any exact way of evaluating the genetic level of the true parents of the children studied in foster homes, and our measures of environmental influences as found in the foster homes and elsewhere leave much to be desired. In general the results of foster child studies show an

increase in I.Q. of about five to ten points when children are shifted from a poor environment into a good foster home. This is a little hard to interpret, however, when we have no really objective yardstick for appraising either the previous environment or that of the foster home. To be sure we may devise scales of environment which appraise some of the objective features of the homes, but we are not at all sure that such scales are getting at the basic dynamic factors in the inter-relationship between child, parents, and other tangible or intangible factors surrounding him, which actually stimulate the development of his mind. Woodworth notes with interest that in the Stanford study of foster children the total influence of home environmental factors upon individual differences in I.Q. scores was estimated as amounting to 17 per cent. This is almost the same as his estimate of 18 per cent reached by another route in evaluating the meaning of the differences in I.Q. of identical twins reared apart. This virtual coincidence of estimates, however, might be due to chance.

Woodworth is somewhat skeptical of all numerical estimates which have been offered regarding the relative potency of heredity and environment. He prefers to treat the problem in terms of recognizable differences in particular aspects of environment viewed as they affect I.Q. differences. This seems to offer no real way at present for ascertaining with any exactitude the contribution of genetic factors to individual differences, unless we are justified in assuming that after all possible environmental influences have been identified, the remainder is the genetic residue. Whether a real appraisal of the genetic contribution must wait upon the identification of the actual genes contributing to differences in intelligence in man, may be impossible to say at present. This reviewer believes that a more careful use of a larger number of cases of twins reared apart may enable the research worker to cut through the entangling environmental obstructions to the actual genetic residue, even if an exact enumeration of the contributory genetic factors is never completely achieved. The fact that several new cases of identical twins reared apart have recently been located or reported on is a hopeful note.

Woodworth includes a careful appraisal of the considerably publicized work done at Iowa University. Here a nursery school experiment was set up in an orphanage where environmental stimulation was very meagre. Children were studied who had had school and institutional experiences of various kinds. He finds the hypotheses and the experiments ingenious, but the evidence disappointing in the extreme, because of the failure of the experimenters to safeguard their conclusions through the establishment of necessary controls, and their failure to consider elementary problems of sampling.

A final section contains suggestions for further avenues of research, a contribution which Woodworth's review monograph would have been incomplete without. Possibly the most interesting is the proposal that identical twins separated at different periods in their development, and even those separated after adulthood, would constitute important material, supplementary to the cases of twins separated in infancy. By such comparative studies, it would be possible to find out at what developmental period of childhood or adulthood certain kinds of environmental influences are effective. The brilliant studies on adult education undertaken in Thorndike's laboratory and elsewhere have shown that adults in general are more plastic than had heretofore been believed. Studies of identical twins separated in adulthood—perhaps entering different occupational fields—would yield data which might go far toward defining the conditions under which various kinds of adult learning takes place. It seems almost self-evident that response to environment never ceases as long as the organism is viable, the problem being not *whether* individuals respond, but how they originally respond, what they respond to, and how experience and growth modify subsequent tendencies to respond.

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BIMODALITY OF CROWDED POLLEN TUBES IN *PRIMULA OBCONICA*

I. MODLIBOWSKA
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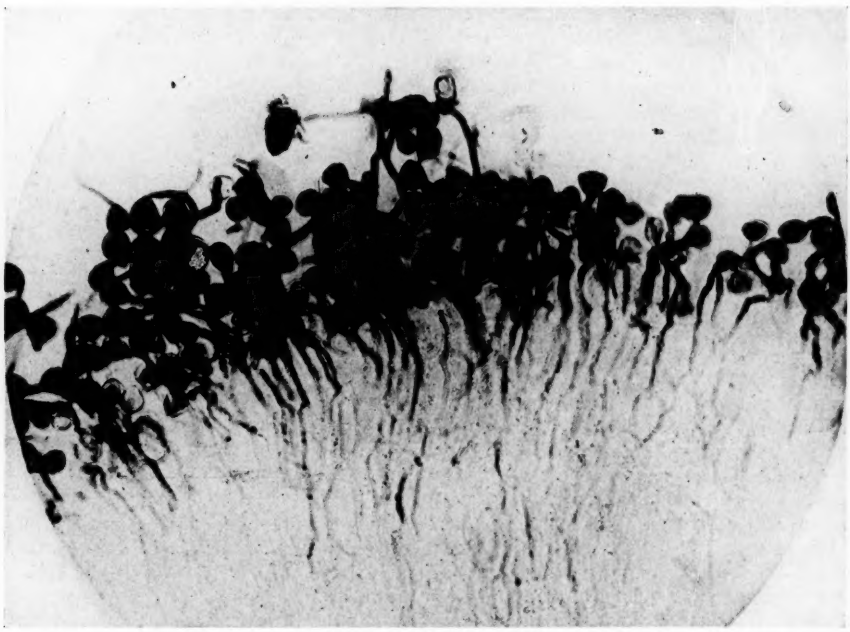

POSITION-EFFECT ON POLLEN GERMINATION
Figure 9

Thrum flowers of *Primula obconica* produce two kinds of pollen, "pin" and "thrum" in equal quantities. When "pin" flowers of *P. obconica* are pollinated with "thrum" pollen, two types of pollen tubes are noted (Figure 10). It might be thought that this is a segregation for the "pin" and "thrum" characteristic, but this is not the case. This photograph shows that the pollen grains in contact with the surface of the style are clear because the cytoplasm has moved out of them into the pollen tube. The germinated grains farther away from the stigmatic surface still are dark. These are the pollen tubes which grow slowly. The bimodality of pollen tube growth is due to crowding, not to genetic segregation of "pin" and "thrum" pollen. Sparsely pollinated stygmas do not show the two modes.

THE flowers of *Primula obconica* are of two kinds, pin and thrum. Thrum is the heterozygote and produces two genetic kinds of pollen. Pin styles are about 6.5 mm. long and thrum styles 3 mm. long. Under suitable conditions, pollination of pin by thrum reveals two types of pollen tubes: long tubes which grow right down the style, and short ones which only penetrate a very small distance.

To discover how this situation comes about, pin flowers of *P. obconica* were cut, put in water and pollinated abundantly with thrum pollen (in *P. obconica* the stigmatic surface is dry even when

receptive). The flowers were incubated at 15, 20 and 30°C respectively and then fixed for five minutes in 3:1 absolute alcohol/glacial acetic acid. The sectioning was mostly done by hand, cutting the style into halves longitudinally with a scalpel. Sections were also made with the freezing microtome. The material was stained with light green and acid fuchsin (Cummings et al[4]) for 30 minutes at about 50°C. The sections were then mounted temporarily in fresh stain, cut surfaces upwards.

Preliminary examinations were made after 6 hours' incubation. At this time practically all the pollen grains in immediate contact with the stigmatic surface had germinated, and had developed tubes about 0.5 mm. long. Other pollen grains, not in immediate contact with the stigma, had also germinated, but their tubes were much shorter, being only about 0.1 mm. long (Figure 9). A layer of grains still further away from the stigma did not germinate (not many of these are seen in the figure, as most of them were washed away by the fixative). The difference in behaviour thus depends on position. Difference in staining also occured at this stage. The ungerminated and short-tube pollen grains stained deeply, but the grains with long tubes did not stain, the nuclei and cytoplasm having passed into the tubes.

In fixations made after 24 hours' incubation at the higher temperatures, the long tubes had grown three-quarters of the way down the style, 4-5 mm, while the short ones remained at the top and did not much exceed 0.5 mm. They formed a conspicuous zone at this level (Figure 10).

It is evident that abundant pollination created different germination conditions as between individual pollen grains, and that pollen less favourably situated on the stigma germinates later and gives rise to short tube growth. This is confirmed by the observation that when pollen is applied sparsely to the stigma, no short tubes occur.

Furthermore, very active pollen was needed to produce the two types of pollen tubes: pollen which had not been kept for more than a fortnight after dehiscence, either on the flower under glasshouse conditions, or stored at 10°C. over $CaCl_2 . 6H_2O$ at about 38 per cent humidity. Pollen from flowers that had been open for more than two weeks, and young pollen stored for the same period but in dry conditions, germinated only when in direct contact with the papillae, and from such pollen no differential tube growth occurred. The age of the styles on the other hand did not play any part in difference in pollen tube growth. Both young and old styles, ranging from flowers in the bud stage up to three weeks old, were used in the experiments, and in all cases long and short tubes occurred when young pollen was applied in excess.

Optimum temperature conditions for pollen tube growth, i.e. 20-30°C., were also necessary for the production of the short pollen tubes. At 15°C. the short tubes were seldom present and if present were mostly unswollen. Therefore the conditions necessary for bimodality of pollen tube growth are:

1. Excessive pollination.
2. Vigorous pollen.
3. Favorable temperatures.

Pollen tube bimodality was also obtained by double pollination—first sparse, then six hours later abundant. 24 hours after the first pollination both long and short tubes were present, just as when pollen was applied abundantly the first time; but in the case of double pollination, very few tubes developed swollen ends.

With the reverse cross, *P. obconica* thrum × pin, short tubes were also present, but in this case the swelling of the tubes was not usually so conspicuous as in the pin × thrum cross.

Pollen tube bimodality has also been found in *Lythrum Salicaria* (Lewis, unpublished) and in *Lycopersicum esculentum* (Modlibowska, unpublished), after abundant pollination. In *L. esculentum* however the short tubes were fewer in number. They were not completely arrested but continued to grow slowly down the style. Since in any case the first lot of tubes does not stain after having reached the ovary, it is easy to over-

TWO "SWARMS" OF POLLEN TUBES

Figure 10

After 24 hours' incubation the long tubes have grown three-quarters of the way down the style (dark core in lower part of style), the short tubes have only grown about a half millimeter below the surface. There they appear as a ragged dark area. Crowding thus produces an effect of partial incompatibility. The elimination of excessive pollen tube competition may be of selective value to the plant.

look the long tubes which have already passed through the style. This may be the reason why Smith and Cochran[6] record that after 24 hours no tubes longer than one-third of the styles were present. I found that during the same period many tubes had already entered the ovary. This is in accordance with Judkins' observations.[5] In *Datura,* Bucholz and Blakeslee have described numerous cases of pollen tube differentiation. According to them it is due to genetic differences in the pollen. Yet these authors admit that some of the short tubes in *Datura* were due to slow germination and to crowding[1] and report to have obtained artificially a bimodal grouping of the pollen tubes by double pollination.[2]

In all my cases the position is simple. Equally in *Primula, Lythrum* and *Lycopersicum,* the style and pollen were genetically compatible. And although the pollen of thrum in *Primula* is of two genetic kinds, it was position and not genetic difference that conditioned the difference in growth. This difference thus appears to be primarily, if not entirely, due to environmental causes, such as overcrowding of tubes in the style and less favourable conditions for their germination and growth. There is however an analogy between these short tubes and those of incompatible crosses. The similarity consists in the slowing down of growth in some cases and almost complete inhibition in others. In my compatible crosses, unsuitable conditions of nutrition seem to be responsible for the inhibition and arrest of growth resulting in short tubes, whilst in incompatible crosses, the genetic incompatibility reaction of pollen and style is the cause.

Up to the present, differentiation of pollen tubes into two classes due to competition has been observed only in species with a non-secreting dry stigma. It is possible that species with a stigmatic secretion offer more uniform conditions for pollen germination. On this view a deficiency of secretion would be a condition of overcrowding and starvation. That the checking of excess pollen tubes occurs in some species and not in others indicates that it may have an adaptive advantage. It is probably better to have good growth of a limited but sufficient number of pollen tubes, rather

than defective growth of an excessive number.

Summary

In *Primula obconica* and other plants with dry stigma, excessive pollination leads to the arrest of late-growing pollen tubes in the top of the style. Thus the effect of crowding is to simulate partial incompatibility.

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SEEDS FOR THE SCORCHED EARTH

A Message from the Seed Committee, Russian War Relief

THE members of the Seed Committee of Russian War Relief (now in process of formation among seedsmen and experimentalists) are eager to have you join the "Plant the Scorched Earth Campaign" now under way to send vegetable seeds to Russia. This campaign has been launched for the purpose of helping to provide the food that will sustain our fighting ally against Hitlerism.

Enough seeds to plant more than 5,000 acres of Russia's soil has been received by Russian War Relief, Inc., since the campaign was launched in March. Contributions of seeds and money earmarked for seeds has been received from every state in the union as well as from Alaska, Puerto Rico, and northern Canada. The seeds will be sent to communities in central Russia and Siberia, where factories have been moved and refugee colonies established, far behind the fighting lines.

The committee is soliciting those seeds (in bulk) which will prove most practical and valuable for planting in Russia. However, some of the plant breeders and agronomists from Experimental Stations have suggested shipping small quantities of pedigreed or first or second generation seeds of new, improved diseases-resistant varieties of other farm crops as well as vegetables. These will be most welcome as a gift to the plant breeders and agronomists of Russia for multiplication of their experimental fields.

Several of the Experimental Stations, among them Cornell and New Jersey, have already signified their willingness to help by sending some of their new strains. Dr. L. H. Newman, Dominion Cerealist of the Central Experimental Farm in Ottawa, Canada, recently wrote us: "This is certainly a worthy undertaking and one with which I would be very glad, indeed, to be associated." Through Dr. Newman's efforts several of the heads of the leading agricultural institutions in Canada have signified their willingness to contribute.

May we have the benefit of your cooperation in passing this information on to your plant breeders and other specialists of your Station. We should like to hear from them what kinds of seeds they are prepared to contribute, and also descriptive material of their origin, etc.

If you would like to have your Station represented on our Seed Committee, we would be happy to hear from you to that effect. Your early reply will be appreciated.

Russian War Relief, Inc.
Seed Committee,
J. W. Pincus, Seed Consultant,
Rm. 309, 535 Fifth Ave., New York City

TYPES OF FUR FIBERS

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WHEN furs of varying qualities are compared, naturally many questions arise, such as: Is the pelt prime? Is the fur dense? How about the guard hair as compared with the underfur? Has it sufficient sheen or does it carry some rust or tinge? If these questions and many others cannot be answered favorably this poses a question for the fur breeder: Where are the deficiencies and what can be done to rectify them in the future?

In the fur trade, the main criterion in judging a pelt is quality, and this is affected by many factors. The pelt must be prime. This may be determined by examining the skin side which, if the pelt is fresh, should be fairly white or cream colored, and show some "life" when handled. The skin side turns yellow and tends to lose its firmness with age. The hair side should be well furred all over, both on the back and on the belly, with the fur of the proper length for the species, — lustrous, and silky. Whether the fur is black or brown, clearness of color is an important factor in determining quality in pelt. Careful buyers make a greater cut in the price of a pelt which has rust or tinge than for any other defect. As a rule more is paid for a clear, bright colored pelt that is not so well furred than for a full-furred skin that has a rusty or faded appearance.

To judge furs satisfactorily requires a great deal of experience and one must handle quantities of furs to become efficient in judging them. To those who are not expert, slight differences seem so subtle that they are hard to understand. The problems which present themselves are: Can physical measurements be obtained which will furnish measuring sticks for primeness, color, luster and general desirability in fur? Considerable research has been carried out upon fur fibers but very little work has been done which assists the fur industry in the analysis and interpretation of merit in commercial furs or serves as a guide in breeding better ones. Breeders of fur animals as well as those who are conducting research work in the field of fur-animal production must be able to recognize quality in furs before we can effectively investigate other factors which may, or may not, be responsible for the production of fur of superior quality.

In an attempt to penetrate more deeply into the mysteries of fur quality the writers took sections of skin from several prime silver fox and mink furs because of their economic importance in fur farming. The general structure of fibers from these animals should be representative of most furs. Fibers taken from these pelts received detailed microscopic study.

Methods of Examination

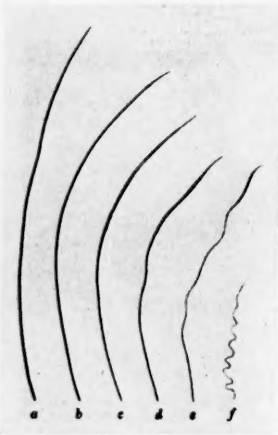
Skin Dissection

Pieces of skin with the fur attached were cut from the choicest part of the pelt, namely the part near the intersection of the dorsal midline and a line drawn immediately in front of the hind legs.

From this small piece of skin tufts of fibers or single hairs were removed, including the roots, by means of a very sharp pointed knife and with the aid of a Greenough type of microscope. Parts of the skin adhering to the root were removed with needles.

Surface Impressions

The surface structure of each of the different kinds of hair found was examined by obtaining impressions, employing the method previously developed by the authors.[2] Difficulty was encoun-



TYPES OF HAIRS
Figure 11
A—Single guard hair (Leithaar); *C*—guard hair; *F*—underfur fiber; *B, D, E*—intermediate forms. After Toldt.

tered at first with some of the coarser hairs because the pressure needed to secure clear impressions often crushed the fibers. To overcome this difficulty a fiber was placed between two pieces of vinylite film 0.005 inch in thickness. On each side of this "sandwich," padding was supplied in the following order: several pieces of thermoplastic film other than vinylite (e.g., ethyl cellulose or cellulose acetate), a glass slide, a piece of rubber sponge tape at least a quarter of an inch thick, and finally a rigid steel slide. The aggregate was held together by a large clamp with sufficient pressure to compress the rubber sponge to approximately half its previous thickness. After leaving this assemblage in an oven at 90°C. for about ten minutes the whole was dismantled and the two vinylite sheets separated while still warm.

Since the vinylite films soften at about 60°C., the fiber sinks into these films rather than into the other thermoplastic films which do not soften except at higher temperatures. The padding used with the other films yields to some extent, and reduces the danger of crushing the fiber. The glass slides have two functions: first, they eliminate the undesirable impressions which the rough surface of the rubber sponge would otherwise transmit through the vinylite films; and, second, they furnish a uniformly flat background without which the tip and sometimes the base of the hairs do not give an impression because of their thinness in contrast with the thickest part of the coarse hair. If the films are left in the oven longer than ten minutes, they are apt to stick together so tightly that they can be separated only in small fragments or not at all.

Mounting Media

It was desired to find media which would be entirely colorless, so that they might be employed in critical studies of pigmentation. The commonly employed medium, Canada balsam, has the disadvantage of possessing a decided yellowish tinge. In order to view the internal structure with a minimum of interference from external reflections, two media having refractive indices* close to that of the fibers were chosen. For temporary mounts tricresyl phosphate ($n = 1.557$ approximately) was used and for permanent mounts polystyrene dissolved in styrene ($n = 1.594$ approximately when dried). When it is desired to view the scales directly, especially in the absence of interfering pigment, media were selected having refractive indices very different from that of the fiber ($n = 1.55$-1.56 approximately). N-butyl phosphate ($n = 1.423$ approximately) (or quinoline $n = 1.620$ approximately, as second choice) is the medium employed for temporary mounts, and acryloid C10 ($n = 1.473$ approximately when dried), for permanent mounts.

The media for temporary mounts are liquids. Acryloid C10 is a very viscous liquid. Polystrene is on the market in the form of crystals under the name Resoglaz. These crystals are brought

*These values were obtained through the assistance of Mr. G. L. Keenan, Microanalytical Division, Food and Drug Administration, and Dr. C. P. Saylor, Chemistry Division, National Bureau of Standards, at their respective laboratories.

into solution by the addition of sufficient styrene to reduce them to the consistency of molasses. The process may be hastened by placing the container on a warming plate maintained at about 50°C.

The exploration of the recently developed plastic materials for different mounting purposes is naturally engaging a number of workers. O'Brien and Hance[3] have just drawn attention to the merits of isobutyl methacrylate as a mounting medium superior to Canada balsam and gum damar. The refractive index of this material was not determined, but examination of fur fibers mounted in isobutyl methacrylate indicates that it approximates that of polystyrene. However, since the refractive index of polystyrene appears to be closer to that of the fur fibers than the isobutyl methacrylate, the former is preferable when it is desired to view the internal structure of these fibers.

When making permanent mounts it is advisable to observe the following precautions. Since both of the plastic media are very viscous, bubbles of air are easily trapped; consequently all stirring must be avoided. Air bubbles are apt also to cling to the fibers. This may be avoided by first wetting the fibers with a very dilute solution of the plastic (ethyl acetate may be used with the acryloid). The diluting medium must, however, be kept to a minimum, otherwise large bubbles of air form under the cover glass while the mount is drying as a result of the appreciable decrease in volume. Such bubbles also form when any attempt is made to hurry the drying by warming. Best results are obtained if the slides are allowed to dry over night under cylindrical weights slightly smaller than the cover glass and weighing 50 to 150 grams.

Permanent mounts may also be prepared without using a cover glass. The fibers are placed on a slide, the plastic solution spread over them, and the fibers moved into the desired position with needles. These are employed also to guide such bubbles as may have been caught, away from the vicinity of the fiber to the edge of the medium. In half an hour at room temperature the film dries sufficiently to permit trimming along the edges and viewing the specimen under the microscope.

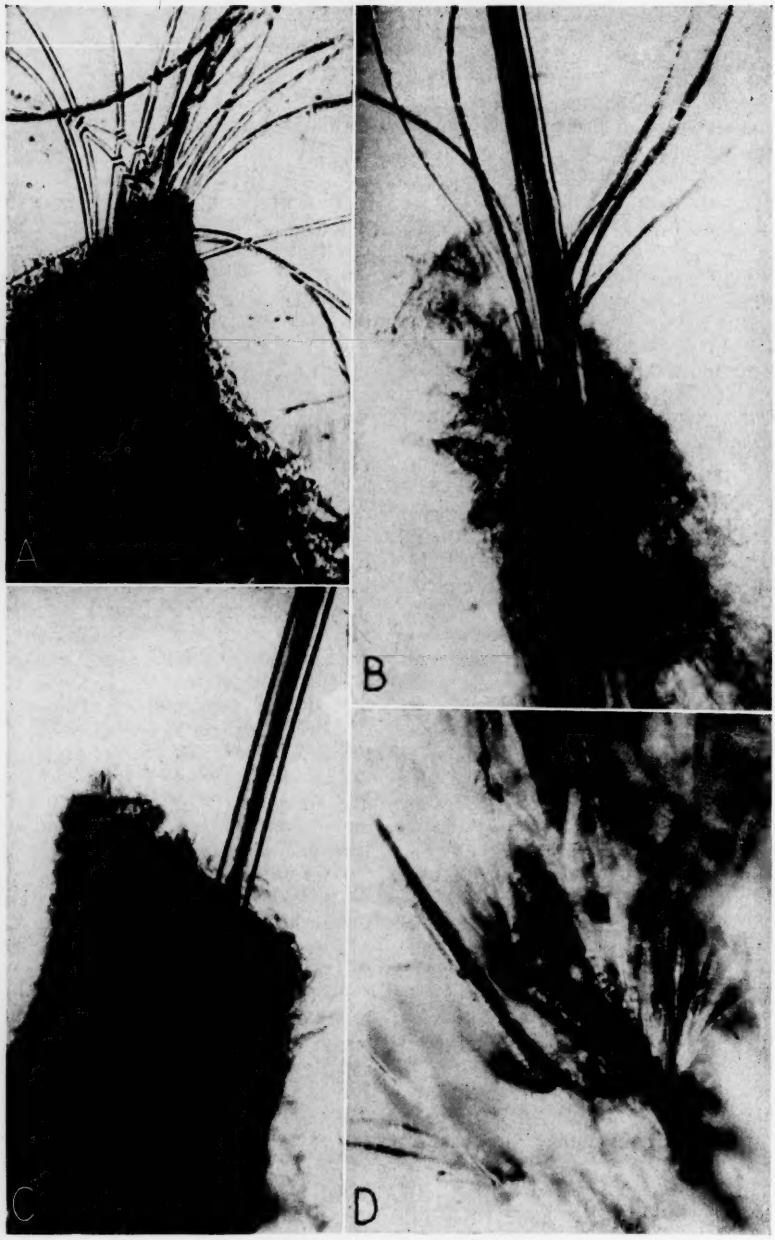
Typical Fibers

Three types of hairs were first recognized by Toldt[5] during the study of the development of hairs in the common European fox. Intermediary forms also exist in both the guard hairs and in underfur so that there is a continuous series (Figure 11). The presence of these intergrading hairs is somewhat confusing; but if a practical grouping of similar hairs is adopted, the difficulty largely disappears. Such a grouping may vary with the needs of the individual, but he will wish to correlate his findings with those reported in the literature. For the writers' purposes three kinds of fibers have been selected that are believed to have practical value in studying the composition and quality of furs.

Those composing the underfur are readily distinguished from the guard hairs. They are usually distinctly shorter and finer and are crimped along most of their length. They issue from the follicles in tufts; this structure is exemplified in the furs of both the silver fox and the mink (Figures 12*A*, 13*A*, 12*D* and 13*D*). The scale structure of underfur, obtained from impressions of fibers from both silver fox and mink pelts, was illustrated in a previous publication of the authors.[2]

Two types of guard hairs have been selected as profitable for study: the more commonly occurring or regular guard hairs (Figures 12*B* and 13*B*) and the less common longer, stiffer, or "single" guard hairs (Figures 12*C*, 12*D*, 13*C* and 13*D*). The regular guard hairs are abundant; they are usually longer than the underfur and cover it; and since a considerable part of these fibers near their tips is enlarged, they protect the underfur from abrasion. Only one regular guard hair issues from a follicle, but this is always accompanied by a number of underfur fibers (Figures 12*B* and 13*B*).

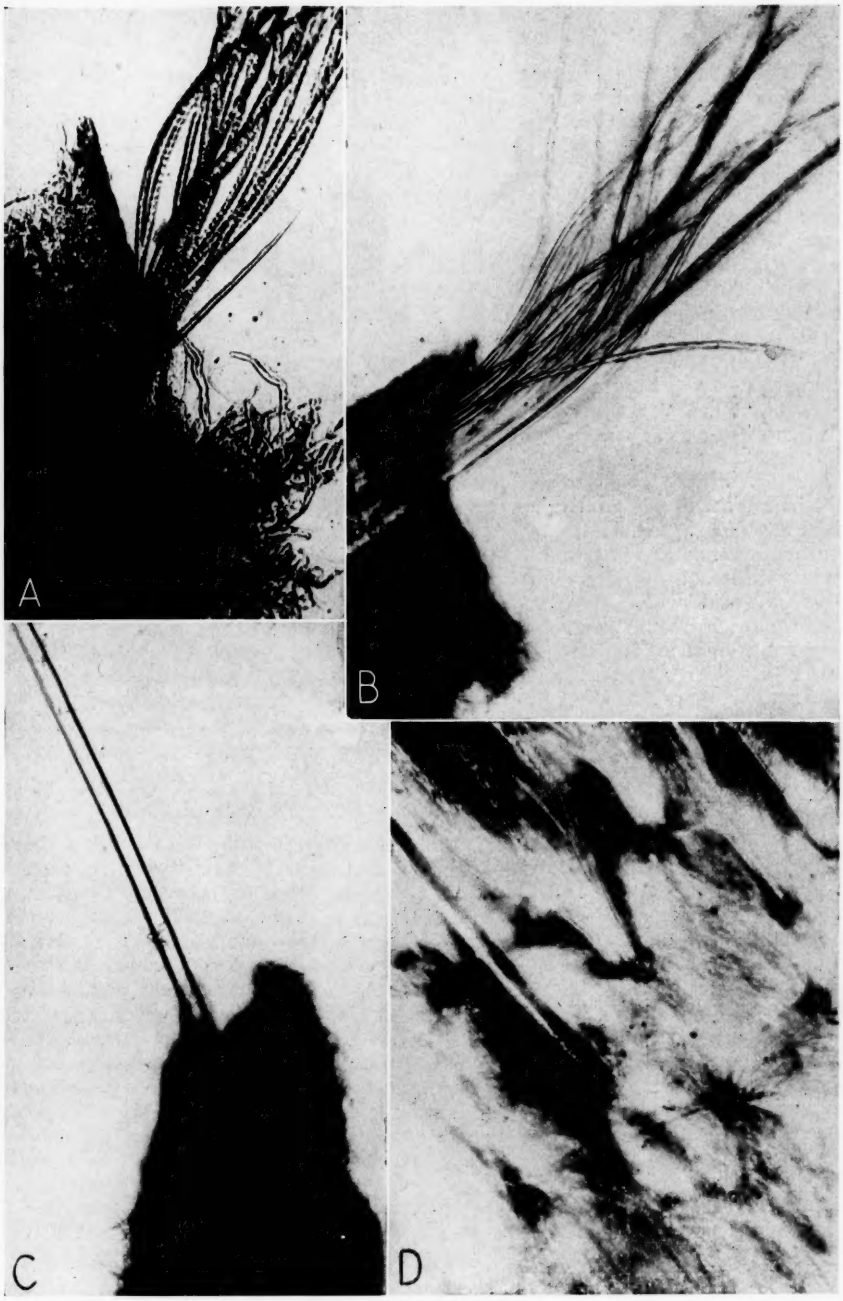
The "single" guard hairs are especially interesting. These hairs were first designated by Toldt[5] as "Borstenhaare,"



SILVER FOX HAIR TYPES

Figure 12

A—A tuft of underfur in one follicle. *B*—A regular guard hair with accompanying underfur fibers in one follicle. *C*—A "single" guard hair in one follicle. *D*—Surface view of a piece of skin showing a single guard hair and tufts of underfur issuing from different follicles. All photographs × 100.



HAIR TYPES IN MINK

Figure 13

A—A tuft of underfur in one follicle. *B*—A regular guard hair with accompanying underfur fibers in one follicle. *C*—A "single" guard hair on one follicle. *D*—Surface view of a piece of skin showing a "single" guard hair and tufts of underfur issuing from different follicles. All photographs × 100.

which he later changed to "Leithaare," (guiding hairs).[6] They are more uniformly stout and stiff than the regular guard hairs, hence the name "Borstenhaare" (bristles). This is probably caused by the comparatively uniform diameter of these hairs, there being less taper at the lower part than in the ordinary guard hair. Toldt discovered them on fox embryos: they are the first hairs to appear and are in rows. Since he believed them to have an important bearing on the arrangement of the fur, he changed the name to "Leithaare."

Some difficulty was experienced in distinguishing these specialized "single" fibers, until some were found with a brown wax-like substance surrounding the hair at the opening of the hair follicle in the dressed pelt. However, subsequent investigations showed that this substance is not always present.

On the back of the silver fox the "single" guard hairs are always black, except for a lightening in color toward the base; the silver bar, so characteristic of the regular guard hairs, is always lacking. These "single" guard hairs are also more nearly straight than the ordinary guard hairs, which are usually strongly bent between positions *b* and *c* (Figure 15), i.e., just below the enlarged upper portion. The very long fine point of the "single" guard hairs is also a distinguishing characteristic. The scale structure, obtained from impressions of both fox and mink fibers, is illustrated in Figure 15.

As shown in Figure 11(*a*), these "single" guard hairs are usually the longest of all the hairs and may often be located by holding the fur at the level of the eyes against a light background. They extend above the level of the regular guard hairs, but are sparsely distributed. Because of this and also because of their peculiar distribution on animals of differing habitats, Toldt[6] deduced that they possess a tactile function. On the fox they are especially abundant on the neck, back, flanks, and at the root of the tail. On the part of the body having shorter hairs the "single" guard hairs are still distinctly longer than the other hairs. On the lower extremities of the body some very long ones may be found.

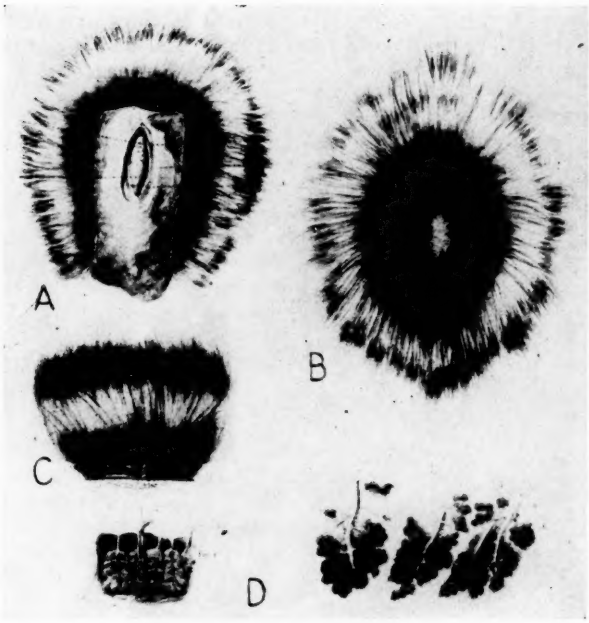
This type of hair has also been found in mice. Dry[1] calls these fibers monotrichs because a hair of this type always stands alone in a follicle, no underfur fibers accompanying them (Figures 12*C*, 13*C*, 12*D*, and 13*D*). Although Toldt[5] mentions their being isolated or accompanied by a few finer hairs, the present study of the silver fox indicates that the "single" guard hair always stands alone in a follicle, whereas those which are accompanied by underfur fibers are regular guard hairs. Williams[7] includes these "heavy overhairs" as one of the three types present in the covering of most mammals.

Plastic impressions of these two types of guard hairs show that the scale structures are quite similar. In the regular guard hairs the transition in scale form from the root to the pattern in position *b* (Figure 15) occurs abruptly; but in the "single" guard hair it is gradual.

Tail Gland Hairs

On the tail of the fox there is a gland first described by the Swedish scientist Retzius.[4] Retzius, according to a translation available to Toldt, described the scent of this gland as being similar to the odor of the roots of violets or rather of the lichen, *Byssus yolithus.** Consequently it was called "Violdrüse" (violet gland). It is located dorsally, approximately a handsbreadth from the root of the tail (Figure 14). In the present examination of the silver fox, on parting the fur in this region, the gland was readily located by the presence of a dense cluster of coarse hairs, which are white from the black tip down to the skin. On an undressed skin which had

*This plant was identified as the lichen *Byssus jolithus* by E. H. Walker, Smithsonian Institution, U. S. National Museum, Washington, D. C. From information procured through J. A. Stevenson, Bureau of Plant Industry, U. S. Department of Agriculture, it appears that this lichen possesses the odor of violets.



DETAILS OF TAIL GLAND

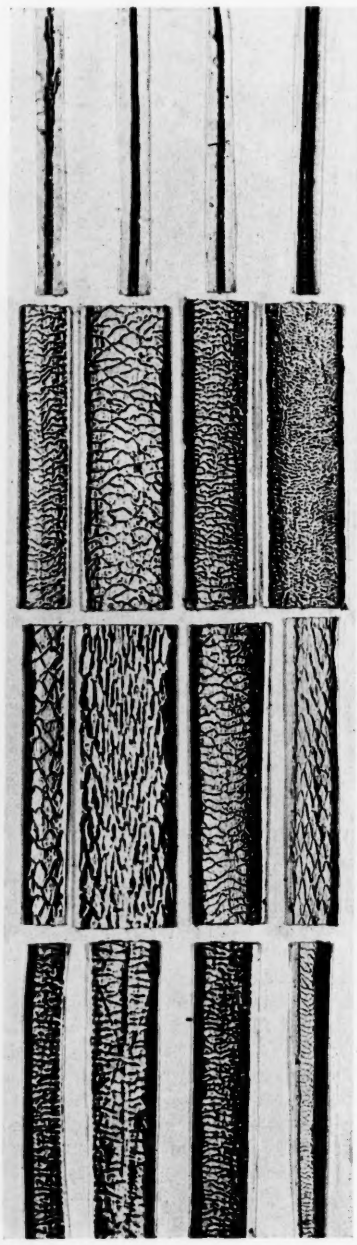
Figure 14

A—The tail gland seen from below, with the skin dissected to expose the glandular tissues. *B*—The same seen from above. *C*—Section showing glandular mass below, also granules of secretion clinging to the hairs. *D*—The secreting glands, greatly magnified. (From Retzius.)

been in storage the odor at this point was offensive. On a dressed skin some remnants of the yellowish exudate were still found but the odor was only faint. Nevertheless, it might be advisable to remove this small glandular area to prevent the fur from giving off a disagreeable odor when exposed to dampness or rain.

Retzius[4] described this spot as lancet-shaped, being 2.5 cm. long, 0.9 cm. wide, and 0.4 mm. deep. He reports that there is no single large gland present; rather it seems that the yellowish fatty secretion is produced by modified sebaceous and sudoriferous glands associated with the follicles in this area. He supposed that the greatest activity of this gland takes place during the rutting period.

The coarse hairs present in this area were apparently considered by Toldt[5] to be "Leithaare" or "single" guard hairs. In the present investigation they were found to be somewhat long with little curvature and rather stiff; moreover, they often stand singly in the follicles. On the silver fox it was noticed that along the periphery of the glandular area there are follicles from which tufts of fibers issue which sometimes include a few underfur fibers; nearer the center were found follicles containing the two stout hairs which Retzius mentioned as standing close together. Hence the coarse glandular hairs may be modified regular guard hairs. The absence of underfur at the center of the glandular area as noted by Toldt[5] has been confirmed. It is conceivable, as that author[5] had pointed out, that the suppression of that type of hair in this region is associated with the development of the coarse hairs and the large glands. It was noted in the present studies that many of the



guard hairs on this glandular area are flattened and distorted, exhibiting large variations in diameter, the constrictions appearing like checks, longitudinal indentations, and even large cracks in the fiber. Such hairs are weak and break readily at the constrictions. As Toldt[5] points out, it seems as if they have been eroded by the glandular secretion.

Impressions of these coarse glandular hairs reveal a peculiar scale structure in position *b* above the root end (Figure 15), the scales are very much elongated and narrow, whereas on the regular guard hairs and the single guard hairs the scales in this position are diamond-shaped.*

Navel Hairs

There is a tuft of coarse hairs near the navel, all of which have black tips, and although most of them are white below this tip, there are others which are dark practically down to the base. Toldt[5] believed these hairs to be "single" guard hairs. However, examination by the present authors reveals that most of the hairs do not occur singly in follicles, but that several coarse hairs, usually accompanied by underfur hairs, emerge from each follicle. The impressions of these coarse hairs (Figure 15), exhibit a preponderant coronal arrangement of the scales throughout the length of the fiber. Thus they differ from both regular and "single" guard hairs, and it seems likely that the common bundles of fibers which issue from a follicle, including a guard hair and several under-

*See illustration in Hardy and Plitt.[2]

SURFACE-STRUCTURES OF HAIRS

Figure 15

Impressions of sections of hairs along their length: *a*—root ends; *b*—a part adjacent to root end; *c*—the widest part of the fiber between sections *b* and *d*; and *d*—the tip of the hair. Hairs *1*, *2*, and *3* are from Silver Fox: *1*—"single" guard hair, *2*—tail gland hair, *3*—navel hair; *4*—is a "single" guard hair from a mink.

fur hairs, have been modified in this small area.

Discussion

New mounting media for the microscopic analysis of animal fibers have been needed for some time. The media here described have several advantages: (a) They are colorless and consequently permit more accurate judging of color values; (b) The refractive index may be varied to bring out either the internal or the external structure of hair; (c) The liquid media facilitate rapid examinations in temporary mounts; and (d) Advantages *a* and *b* also characterize permanent mounts.

It is believed that recognition of the three main types of fibers, the underfur fibers, the regular guard hairs, and the "single" guard hairs, is fundamental to the further analysis of the composition of furs with respect to variations in quality.

Summary

For the microscopic examination of fur fibers two colorless media (synthetic plastics) have been found useful for permanent mounts, and two liquids for temporary mounts. Study of the pigmentation or of the cuticular scales may be favored by choice of refractive indices.

Microscopic examination of prime silver fox and of mink pelts led to the conclusion that three main types of fibers contribute materially to the quality of furs. These are the underfur fibers, the regular guard hairs, and the "single" guard hairs. The three types of hairs were studied for general characteristics, such as relative lengths, form, and color, as well as specific characteristics, such as the features of their surface structure and relations to each other in the follicles. Specialized hairs are present in the tail gland and in a tuft near the navel.

Impressions of the surface structure of the fibers were made on thermoplastic films by a modification of the original method. This modification was necessary to prevent the crushing effect on the thicker fibers. These impressions reveal features of surface structure in certain parts of the hairs that are characteristic for certain types of fibers and for different species of animals.

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COLCHICINE STIMULATION OF SEED GERMINATION IN *PETUNIA AXILLARIS*

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SEED treatment is probably the simplest of the many methods by which colchicine has been applied to plant parts for the production of polyploidy. Few of the methods thus far reported are suited to use in treating large quantities of material.[2] Soaking seeds in aqueous solutions has the advantages of requiring no special equipment and of using relatively small amounts of solution. Most investigators, however, have found this otherwise promising method unsatisfactory because of poor germination of the treated seeds. *Datura* seeds soaked in solutions of colchicine, in concentrations ranging from 1.6 to 0.003125 per cent germinated well.[1] On the other hand, soaking strawberry seeds in colchicine solution for nine days was completely lethal.[3] Germination of treated seeds of *Petunia*[4] and of perennial ryegrass[5] was very poor. The immediate effects of colchicine treatment of seeds were reported as a delay in germination, a reduction of the total number of seedlings, and the production of abnormal, slow-growing seedlings.[1,5] The germination of exceptionally large numbers of colchicine treated *Petunia axillaris* seeds, from which polyploid plants were obtained, was, therefore, unexpected and seemed worthy of further investigation.

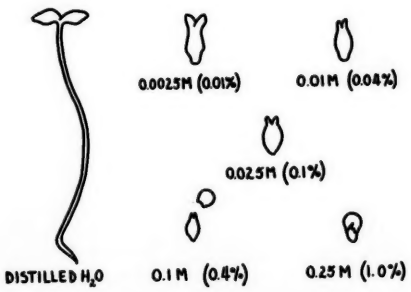
The *Petunia axillaris* seeds whose germination was first noted as unusually good were being treated in a series of distilled-water solutions of colchicine (Merck) in the following concentrations: 0.01, 0.04, 0.1, 0.4, and 1.0 per cent (expressed in molarity, 0.0025 M, 0.01 M, 0.025 M, 0.1 M, and 0.25 M). One hundred fifty seeds were soaked in each solution, and a control lot was soaked in distilled water. Fifty seeds from each lot were planted after two days, fifty more after five days, and the rest after nine days. At the end of five days, many seeds had germinated in the solutions. A closer examination revealed that the number of seeds germinated in each solution was different, and that the form of the protruding seedling was not the same in all cases. The highest germination was found in the 0.04 per cent solution. The seeds that were planted after two days' soaking showed no germination at the time of planting. The effect of the colchicine on germination was evident, however, in the number of established seedlings obtained from each of the lots of fifty seeds. From those soaked in distilled water, twenty-seven seedlings came up; from the lots soaked in the series of colchicine solutions, from weakest to strongest, 39, 45, 42, 37 and 22 seedlings, respectively, came up. Here again, highest germination followed treatment in 0.04 per cent colchicine. There were polyploid plants among the seedlings grown from these seeds.

Another series of treatments was made specifically to determine the effect of colchicine on seed germination. Seeds, all from one capsule, were counted, and lots of one hundred were placed in fresh aqueous solutions of colchicine in the se-

TABLE I. Germination percentages of *Petunia axillaris* seeds in distilled water and in colchicine solutions.

Concentration of Colchicine in Solution	Per Cent Germination in Solution
0.01	98
0.04	100
0.1	88.5
0.4	78
1.0	67.5
water	52

*Formerly Assistant in Botany, Wellesley College, Wellesley, Massachusetts.



CONTROL AND TREATED SEEDLINGS
Figure 16

Typical fifteen-day seedlings, showing the effect of colchicine solutions on growth and development. Colchicine in weak solutions increased the germination rate but reduced the rate of growth during early stages.

ries of concentrations given above, with one lot in distilled water. The seeds that had germinated after soaking seven days were counted. The germination percentages are presented in Table 1. The germination percentage in even the strong colchicine solution was greater than that in water. In the weaker solutions, nearly all of the seeds germinated.

In addition, two more lots of one hundred seeds each, from the capsule used in the previous series, were soaked, one in water redistilled from Pyrex, the other in 0.04 per cent colchicine in redistilled water. There was 99 per cent germination in the colchicine solution compared to 61 per cent germination in the redistilled water. This shows again that the colchicine increased the number of seeds germinated. The lower germination in the distilled water than in the redistilled water may have been due to the presence of toxic ions.

The stimulating effect of the colchicine was not only to increase the number of seeds germinated, but also to reduce the germination time. In the 0.04 per cent colchicine solution in redistilled water, the time required was three days, a whole day less than in the redistilled-water control.

In the observations of germination, seeds in which the radicle had broken through the seed coat were counted as germinated. The effect of the colchicine upon the subsequent growth and development of the seedlings was very marked, as is shown by outline scale drawings of typical seedlings that had been in the solutions for fifteen days (Figure 16). The hypocotyls were enlarged in a way typical of seedlings grown from treated seeds. Chlorophyll did not develop in the cotyledons of seedlings in the colchicine solutions stronger than 0.04 per cent. The effect of colchicine was strongly to inhibit growth of the emerging seedlings. After treatment in colchicine solutions stronger than 0.1 per cent, the number of surviving seedlings was reduced.

Summary

The germination of *Petunia axillaris* seeds was stimulated by colchicine. There was an increase in the number of seeds that germinated as well as a reduction in germination time. The optimum concentration for germination (0.04 per cent) was high enough to induce polyploidy. Growth of seedlings was seriously retarded only by colchicine in concentrations of 0.1 per cent or stronger, the concentrations that have been most widely used in seed treatment. In view of these results, it seems that seed treatment using weak solutions of colchicine rather than strong, the optimum concentration to be determined for each kind of plant, should yield satisfactory results in the practical production of large numbers of polyploid plants.

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A NEW TYPE OF AUTOSOMAL NAKEDNESS IN THE DOMESTIC FOWL

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THE types of genetic nakedness that have been reported in birds are of two categories: (1) those in which the nakedness is due wholly to some abnormality of the feather follicles with normal pterylosis; (2) those in which the nakedness is due to the absence of pterylae. Examples of the former are the "porcupine pigeons" of Cole and Hawkins,[2] the generalized nakedness of the fowl reported by Hutt and Sturkie[7] and the flightless condition in the fowl described by Warren.[12] The latter category includes the "bare neck" character reported by Greenwood,[5] congenital baldness described by Hutt[6] and Jaap[8] and the new type of nakedness herein reported in detail, and elsewhere in brief.[11]

Description of Character

The character varies in expression from an extreme degree of nakedness, although not complete, to almost normal. Moreover, the nudity is due wholly to the absence of pterylae in the areas of the body concerned. Where follicles are present, feathers develop normally. Down and semi-plumes are absent in all apteria (Figure 17).

The tracts most commonly absent or greatly reduced in size are the anterior and posterior spinal tracts, and the humeral and the femoral. There is present in the head tract an almost normal complement of feathers except for the area over the eyes which is usually denuded. The ventral tracts of the extreme naked chicks are usually absent or small; however, in chicks showing the less extreme degrees, these tracts may be only slightly reduced in size. One of the wing tracts, the alar, is always normal, while the crural or leg tract is only partially devoid of feathers, or in some cases normal. The degrees of nakedness observed can be assigned to four arbitrary groups or grades. The grading is based upon a general lack of feathers extending to all tracts of the body, rather than upon degree of feathering in any particular tract or tracts. For this reason, only three of four grades are shown in the photographs (Figure 17), since it is difficult to show the four grades from any one view. In accordance with the scheme, employing four degrees, the grades of nakedness shown in Figure 17 are IV, II+ and I, in descending order of degree of denudation.

The results from most of the crosses show a preponderance of individuals belonging to grades II and III. The less frequent grades, IV and I occur in the order named. Details concerning grade frequencies in the progeny of individual males are presented in a later section.

Genetics of the Character

A uni-factorial dominant. The character was observed in one Rhode Island Red chick out of 5,000, hatched from non-pedigreed matings in the autumn of 1939. With especial care, this chick, which fortunately proved to be a male, was raised and was subsequently mated to normal females. From these matings 335 naked and 348 normal chicks were produced. Likewise, heterozygous males from the succeeding generation have been mated to normal females; resulting in a ratio, with the above totals included, of 623 naked to 616 normal individuals. This is obviously a close fit to the expected 1:1 ratio for a simple dominant character.

Not sex-linked. From a mating of F_1 naked females to a normal male, a ratio of 1:1 for naked males (8) and naked females (7) was obtained, thus proving the mutant to be a simple autosomal one. Were the character sex-linked, all of the

naked chicks from this mating would have been males.

F_2 ratios. Because of the high mortality in the growing stock, few laying naked females have been produced. However, from one F_2 mating involving only a few naked females, a ratio of 89 naked to 27 normals was obtained. This is not a significant deviation from the expected 3:1 ratio.

In keeping with the expected ratios, one-third of these 89 naked individuals should be homozygous for the character. Such homozygotes have not, as yet, been segregated and tested directly for degree of nakedness, livability, feed consumption and growth as have the heterozygotes. After examination of the data on these naked F_2's, relative to the above characteristics, it appears that the effect of the gene in homozygous form is not different from that in heterozygous form.

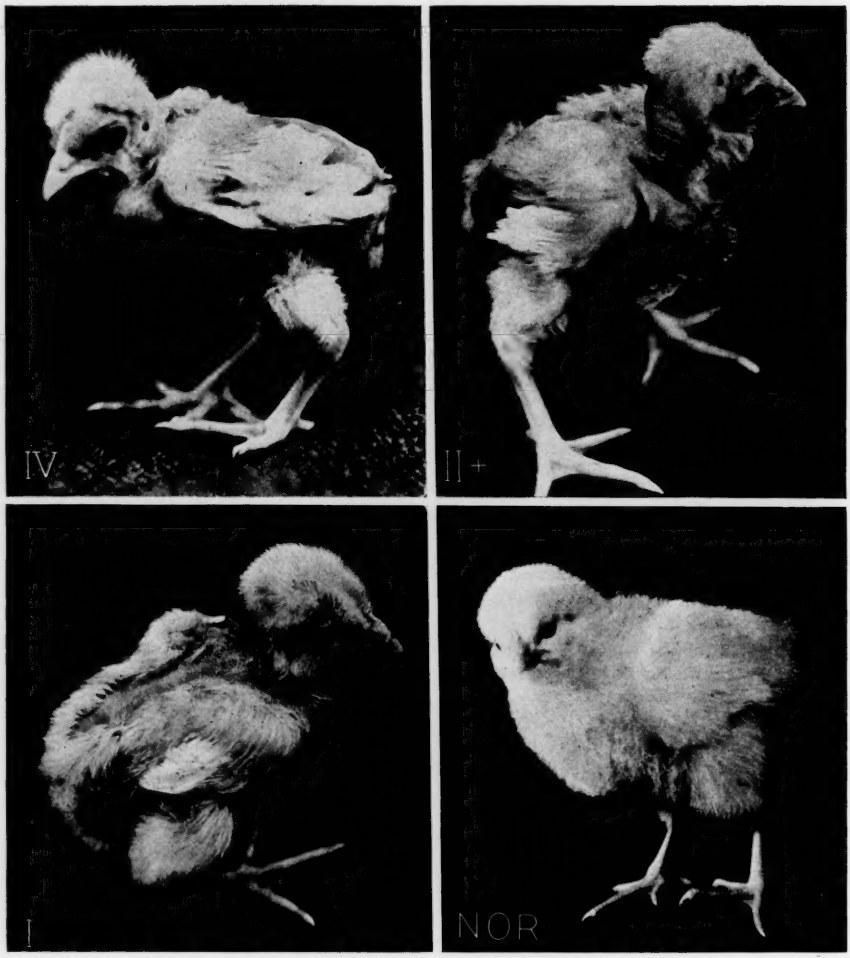
Origin of mutation. As stated above, the character was observed in only one chick out of 5,000 hatched from nonpedigreed matings. Moreover, there was no record of the occurrence of this character in previous generations of this flock. Actually the eggs for the various settings came from approximately 250 breeding females and these hens were mated with 20 males; four males to each pen of 50 hens.

The evidence is in favor of the view that the mutation is recent, most likely having its origin in the immediate male or female parental germ cells. That it occurred in the female germ cells rather than the male is more likely for the following reason. If the mutation originated even as early in gametogenesis as the primary oöcyte stage, at most only one mutant ovum could result in a given female, while in spermatogenesis more than one mutant germ cell would be expected from a primary spermatocyte. Since each male had opportunity to mate with 50 females, assuming random mating, the chances of producing more than one naked progeny from such distribution of male germ cells are greater than for the female germ cells. Such would not be the case, however, if it is assumed that the mutation occurred in one spermatid or spermatozoon, an assumption which seems less plausible to the author than the preceding one.

Modifiers of Dominance

According to Fisher's[4] theory of dominance, under natural selection, modifiers tend to accumulate against dominant characters in the fowl. Modifiers of dominance have been shown to operate in the fowl by other workers; particularly is this true concerning characters normally exhibiting a wide range in variation of expression. The naked character, which also exhibits this range in variation, has been transmitted through only two generations of normal stock; therefore, the data relating to this point are rather meager. Nevertheless, if modifiers of dominance have developed during this time, they should be evidenced by a relative increase in the number of progeny showing the less extreme grades of the defect, particularly grade I.

In Table 1 may be observed the frequencies of the different grades of the anomaly in the progeny from matings of heterozygous sires to normal females (an average of ten females mated to each sire). It is noted that sires *B*, *C*, and *D*, produced greater numbers of chicks belonging to grades III and IV, whereas sire *A*, the original mutant male and sire of *B*, *C*, and *D*, produced more progeny of grades II and III. Contrariwise, male *E* (grade I) sired more offspring belonging to grades I and II than to the other grades. This male's dam, although mated to sire *A*, hatched chicks all of which were grades I and II, differing greatly in this respect from the average female of this mating. It appears that the dam of male *E* transmitted modifiers of nakedness to her son, and that he in turn passed on such to his progeny. No other males from this dam survived to be tested. In the two generations thus observed, natural selection for modifiers toward the normal condition has apparently been without effect. But the evidence concerning the grade I male indicates that modifiers could be accumulated by artificial selection.



GRADES OF NAKEDNESS IN CHICKS

Figure 17

Naked chicks are classified in four grades of which three are shown here, as compared with a normally feathered chick. Specific areas of the body are affected, and the alar wing tract is always nearly completely feathered.

Other Effects of the Gene

Lethal Effect

There is a degree of lethality associated with the character in hatched chicks, but the embryonic mortality is no higher than in normal chicks. The hatchability percentages of all fertile eggs, producing naked and normal chicks were 87.6 and 90.0 per cent respectively. Moreover the naked chicks appear normal in other respects when hatched. The weights of day old naked chicks were normal, and likewise their levels of hemoglobin. Postmorten examination of the naked individuals revealed nothing abnormal anatomically.

In the course of the experiments, it was soon discovered that a high per-

centage of naked chicks died at 10-15 days after hatching. In an attempt to minimize the mortality, special procedures of feeding and management were employed. The naked chicks were brooded separately and at slightly higher temperature than normals. Furthermore, some of them were fed wet mash for certain periods of time, while others were allowed to feed on dry mash, a practice commonly in use. In most cases, the normal sibs were used as controls.

Mortality of naked and normal chicks on dry mash. It was found that the mortality in the naked chicks was considerably higher than for normal chicks throughout life, but that the highest death rate occurred during the first 10-15 days of life (Table II). The figures shown in the table are based on 30 different hatches. While the number of chicks dying in the different hatches varied a great deal, the percentages of mortality for all naked chicks on dry mash were 54.1 at ten days of age and 57.9 at fifteen days, as compared to 2.1 and 2.7 for the controls. The death rate from fifteen days on, declined relatively but still was higher than that for the controls. By 150 days of age, 78.0 per cent of the naked chicks were dead, as compared to 23 per cent for the controls.

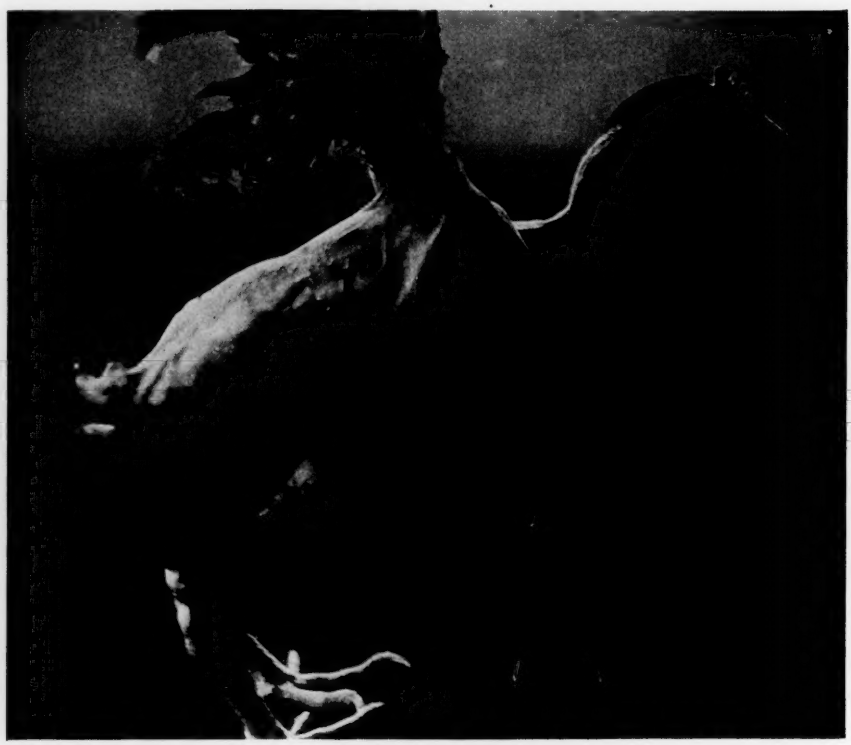
Mortality of naked chicks on dry and wet mash. The data in Table II also show the effects of feeding wet mash on the rate of mortality. It is observed that when naked chicks were fed wet mash for the first ten days of life, the mortality at the termination of this period was only 22.2 per cent as compared to 54.1 for those on dry mash. When this group was then placed on dry mash, the death rate at the succeeding stages shown in the table, increased relatively more than was observed for those on dry mash alone. Thus it appears that feeding wet mash for ten days mitigated the lethal effect of the character, but afforded no permanent protection, since after removal of wet mash, the mortality increased relatively more, and by 60 days of age it was not significantly different from that of the dry mash group. It should be pointed out, however, that there was some variation in regard to the response of the various hatches on wet mash, as shown in Table IV of a later section.

Naked chicks on wet mash for 30 days. The mortality for this group at ten days of age was of the same magnitude as that for the ten-day wet mash group, but at succeeding stages up to and including thirty days of age, it was lower than observed for those fed wet mash only ten days. After this time (on dry mash) the increase in mortality was relatively more, though little more, than in the other naked groups. These results indicate that wet mash, at least for the time used, provides a degree of protection by deferring death. Whether or not the protective effect of the wet mash could be maintained by feeding it continuously has not been adequately determined. The impression is that such protection is probable, although not to any appreciable degree.

Mortality as related to degree of nakedness. It has been demonstrated in various organisms including the fowl that heat loss is, in part, dependent upon the amount and type of covering of the body. Therefore it is expected that the heat loss in the naked chicks would be more extensive than in normal chicks. If this factor or others consequent to this one, figured significantly in the excessive mortality, one might expect to find a difference in mortality of the various grades of naked chicks. Particularly should this be true of grades I and IV. Mortality figures for the different grade chicks at ten days of age when fed dry mash are shown in Table III. It is evident therefrom that degree of nudity is not causally related to death rate.

Feed and Water Consumption

It is generally known that feed consumption of chickens can be increased by wetting the feed. Since on wet mash, the mortality of the naked chicks was less than when on dry mash, the possibility existed that those on dry mash did not consume adequate quantities of feed. Experiments were initiated to test this possibility. Table IV gives the feed and water consumption, and the mortality at



EXTREME NAKEDNESS

Figure 18

Even in grade IV fowls the alar tracts of the wing and part of the legs are fully feathered, the rest of the body, except the top of the head being almost completely bare. Naked is a semi-lethal in the sense that naked birds while hatching in the same percentage as normal, show high mortality during the early stages of life.

ten days for the naked chicks on wet and dry mash, and likewise, the same for normal chicks. The feed and water consumption figures were computed on the basis of per chick per day. It is noted that the average consumption of dry mash per naked chick per day based on the ten day period was 6.3 grams. The figure for the normal chicks on dry mash was 9.8 grams. This latter figure, while based on only two determinations, is in agreement with that of Charles and Knandel[1] who found that normal chicks, based on first seven days of life, consumed 9.7 grams per chick per day.

The water consumption of the naked chicks appears to be normal. These data, while not extensive, show that the naked chicks consumed about one-third less feed per day than normal chicks, when both were on dry mash, and that the degree of protection afforded by the wet mash is, in general, due to a greater consumption of feed. In most of the groups observed in the table, mortality was negatively correlated with food consumption; however, there were exceptions. It has been revealed that the peak of mortality comes within the first ten days, in most cases, while exceptional cases exhibit the peak from 10-15 days, and this fact may account for the discrepancies.

Growth and Reproduction

That the rate of growth of naked chicks is less than that of normal chicks

is indicated by the body weight (in lbs.) at the various ages (in weeks) as follows:

Weeks	*Naked*	*Normal*
3	.178	.223
4	.348	.400
6	.500	.550
8	.620	.870
11	1.39	1.56
16	2.25	2.50
24	3.3	4.4

These weights, for the most part, are based upon a small number of individuals; not more than ten nor less than five, and on the weights of females after six weeks except at 16 weeks where weights of males and females for both naked and normal are included. The weights of the naked birds are consistently lower than those of normal birds at these ages. The weights at 24 weeks of age are based on ten naked (five Reds and five Red-Leghorn hybrids) and 30 normal Reds. Even at one year of age the nakeds (seven individuals) averaged only 3.6 pounds.

The average age at which the ten naked females reached sexual maturity as measured by age at laying of first egg was 182 days, roughly the same as that for normal Rhode Island Reds. The egg production of the nude females is normal, when the birds are in a good state of health.

Discussion

Interest attaches to the lethal action of the gene. Evidence has been presented to show that high death rate was not due to the physical effects resulting from the absence of feathers. It was demonstrated that the lethality was associated with a lowered feed consumption; however, this appears not to be the primary cause of death, but rather a consequence of some basic physiological disorder or deficiency, possibly one concerned with digestion and assimilation. The disorder may be one similar to that reported by Crew[3] for the rat, (with normal pelage) wherein it was shown that the young developed some abnormality at 9-12 days after birth, and although fed, lost weight and in most cases died within five days after the onset of the disturbance. He believed that death resulted from inanition but was unable to determine the primary cause of it.

Hereditary nakedness in its various forms has been reported in a number of mammalian species and in the fowl. There may be found in these classes examples of the association of lethality with the defect. Notable ones are: the hairless condition in cattle, reported by Mohr and Wriedt[9] and also others, hairlessness in the rat, and other types of nakedness in the domestic fowl. In the hairless Norway rat, Roberts et al.[10] have shown that length of life, growth rate, and fertility are reduced. In the sex-linked type of nudity reported in the fowl by Hutt and Sturkie,[7] embryonic mortality as well as that in hatched chicks was higher than in normal chicks. Likewise the restricted type, flightless in the fowl (Warren[12]) appears to reduce viability, particularly in those homozygous for the defect.

Summary

A new type of nakedness occurring in the fowl is described. The character varies in expression from an extreme degree of nakedness, although not complete, to almost normal. The nudity is due wholly to the absence of pterylae in the areas of the body concerned. Where follicles are present, feathers develop normally. Down and semiplumes are absent in all apteria of the body.

The character is inherited as a simple autosomal dominant one. The evidence is in favor of the view that the mutation is recent, most likely having its origin in the immediate female parental germ cell. The data indicate that genetic modifiers of the character toward the normal have not developed in two generations of transmission of the character.

There is a degree of lethality associated with the character in hatched chicks, but the embryonic mortality is no greater than in normal chicks. The mortality is high at all stages of life, but particularly so during the first fifteen days, running as high as 57.9 per cent during this period. The lethal action of the gene is mitigated by feeding wet mash for certain

periods of time; however, after discontinuance of wet mash mortality increases considerably.

The feed consumption of the mutant chicks on dry mash is about one-third to one-half less than that of normal chicks, while on wet mash it is increased; but it is still below normal.

The body weights of naked birds are also below those of normal ones at all weight periods except at hatching time; even at one year of age body weight is still below normal.

Evidence is presented to show that high death rate is not due to physical effects resulting from lack of feathers. While it is demonstrated that lethality is associated with a lowered feed consumption, this appears not to be the primary cause of death, but rather a consequence of some basic physiological disorder, possibly one concerned with digestion and assimilation.

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TABLE I. Frequencies of grades of nakedness.

No., grade & generation of naked sires	No. of progeny	Grade frequencies in percentage I	II	III	IV
A--III -P_1	395	9.8	41.6	37.6	11.0
B--III--F_1	118	5.0	19.5	26.3	49.2
C--III--"	112	9.1	25.8	25.0	40.1
D--II---"	92	10.5	18.4	38.5	32.6
E--I----"	176	63.6	23.8	9.8	2.8

TABLE II. Mortality of naked fowl.

	Normal Chicks (Sibs of naked) Dry Mash	Naked Chicks Fed: Dry mash	Wet mash for: 10 days	30 days
No. Started	330	221	118	99
Mortality in percentage at:				
10 days	2.1	54.1	22.2	21.2
15 "	2.7	57.9	31.3	28.0
30 "	5.8	64.2	52.5	37.4
60 "	9.4	71.9	68.6	51.5
150 "	23.0	78.0	--	67.0

TABLE IV. Feed in water consumption of naked and normal chicks. Feed in grams and water in c.c. per chick day.

	Naked Chicks Lots 1	2	3	4	5	Mean	Normal Chicks Lots 1	2	Mean
On Dry Mash									
No. Chicks	15	18	7	11	14	-	23	14	
Feed	5.7	5.3	5.2	5.1	10.4	6.3	9.6	10.1	9.8
Water	-	-	-	13.0	12.0	12.5	-	12.4	
Mortality in %, 10 days	47	67	29	18	14		0	0	
On Wet Mash									
No. Chicks	22	10	12	11			11	10	
Feed	6.5	8.7	13.3	6.0		8.6	13.5	8.8	11.1
Water	11.7	14.7	-	16.5		14.3	-	12.9	
Mortality in %, 10 days	37	50	0	0			0	10	

TABLE III. Mortality as related to degree of nakedness.

Number started (S) and Dead (D) at ten days.

Grades	I S	D	II S	D	III S	D	IV S	D	ALL S	D
Group 1	22	9	104	31	58	19	30	18	214	77
" 2	70	49	26	22	7	7	3	2	106	80
Totals	92	62	130	85	65	46	33	14	320	157
Per cent dead		67.4		65.4		70.7		42.4		49.0

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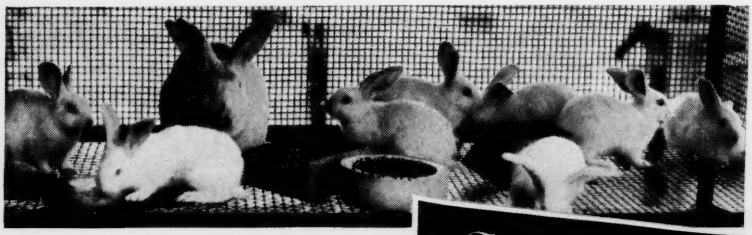

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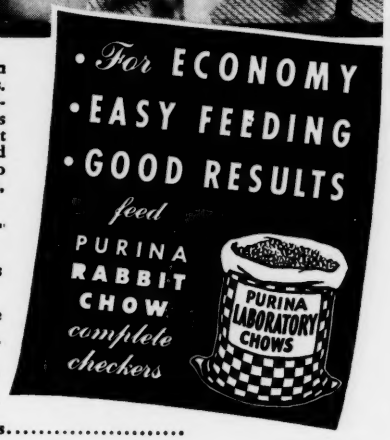

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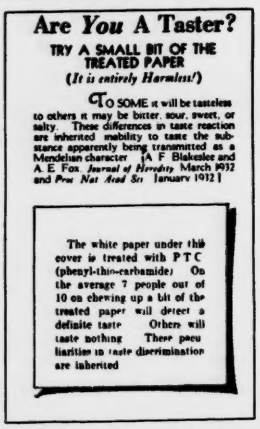
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